 doi:10.1093/bfgp/els049

Linking micro- and macro-evolution at the cell type level: a view from the lophotrochozoan *Platynereis dumerilii*

Oleg Simakov, Tomas A. Larsson and Detlev Arendt

Advance Access publication date 20 November 2012

Abstract

Ever since the origin of the first metazoans over 600 million years ago, cell type diversification has been driven by micro-evolutionary processes at population level, leading to macro-evolution changes above species level. In this review, we introduce the marine annelid *Platynereis dumerilii*, a member of the lophotrochozoan clade (a key yet most understudied superphylum of bilaterians), as a suitable model system for the simultaneous study, at cellular resolution, of macro-evolutionary processes across phyla and of micro-evolutionary processes across highly polymorphic populations collected worldwide. Recent advances in molecular and experimental techniques, easy maintenance and breeding, and the fast, synchronous and stereotypical development have facilitated the establishment of *Platynereis* as one of the leading model species in the eco–evo–devo field. Most importantly, *Platynereis* allows the combination of expression profiling, morphological and physiological characterization at the single cell level. Here, we discuss recent advances in the collection of –omics data for the lab strain and for natural populations collected world-wide that can be integrated with population-specific cellular analyses to result in a cellular atlas integrating genetic, phenotypic and ecological variation. This makes *Platynereis* a tractable system to begin understanding the interplay between macro- and micro-evolutionary processes and cell type diversity.

Keywords: *Lophotrochozoan; micro-evolution; macro-evolution; Platynereis; cell type; comparative genomics*

INTRODUCTION

Understanding the ancient origin and subsequent evolutionary diversification of cell types is one major yet poorly understood area of evolutionary biology. Ever since the beginning of metazoan evolution, animals explored new types of ecological networks (such as simple predator–prey interactions) [1]. Body plan and cell type diversification has been governed by environmental interaction and population variation (i.e. micro-evolution), ultimately resulting in long-term changes in the genomic landscape and phenotypes of a given species over evolutionary time (i.e. macro-evolution). The scope of this review is to explain how the study of both micro- and macro-scales can be combined in the genetic, morphological and physiological characterization of cell types in the marine annelid *Platynereis dumerilii*, a nereidid errantian annelid [2]. We will first describe *Platynereis* as an established system for comparative cell type studies across bilaterians, followed by the recent advances in the analysis of its natural populations and their genetic, genomic, as well as phenotypic and ecological variation. Then, we will explain techniques and resources currently available for the system and finally how they all can be integrated to produce a high-resolution cellular model of macro- and micro-evolutionary cell type diversification.

Corresponding author. Detlev Arendt; EMBL Heidelberg, Meyerhofstr. 1, 69117 Heidelberg, Germany. Tel: +49 (0) 6221 387-8624 Fax: +49 (0) 6221 387-8166. E-mail: arendt@embl.de

Oleg Simakov is a PhD student at EMBL, Heidelberg, Germany.

Tomas Larsson obtained his PhD from the Department of Neuroscience, Uppsala University, Sweden and is currently a postdoc at EMBL Heidelberg.

Detlev Arendt obtained his PhD in zoology in 1999 from the University of Freiburg, Germany. In 1999, he joined the lab of Joachim Wittbrodt at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany. After 3 years of postdoctoral training, he set up his laboratory at the EMBL in 2003 as a Group Leader in the Developmental Biology Unit. In 2007, he became Senior Scientist at the EMBL and was awarded an honorary professorship at the University of Heidelberg, Germany.

THE LOPHOTROCHOZOA: A DIVERSITY STILL TO BE UNCOVERED

The lophotrochozoan superphylum is one of the still observable remnants of an ancient bilaterian diversification referred to as Cambrian explosion [3–5]. Having been recognized only recently as a separate superphylum [6, 7], lophotrochozoans represent one of three major bilaterian clades (Figure 1B). Despite this, relatively little is known about their biology and evolution, compared with their deuterostome and ecdysozoan counterparts. Lophotrochozoans predominantly inhabit marine environments and play a major, and arguably ancient, role in their ecological networks [9]. There are also some clades (e.g. some oligochaetes) that specialized to live on land and a few representatives colonizing freshwater (e.g. the flatworm *Schmidtea*, or even within the mostly marine *Nereididae* family, *Nereis limnicola* [10], which suggests that freshwater adaptation arose independently several times). The original naming [6] of the clade comes from the characteristic lophophore present in, e.g. bryozoans and phoronids, and the stereotypic trochophore larvae with ciliary bands (trochs). Morphological diversity of larval and adult forms is large in this clade, and especially pronounced for adult forms that are as diverse in form as flatworms, bivalves and cephalopods.

Despite being one of the most specious and morphologically diverse clades of bilaterians [11], little is known so far about lophotrochozoan genomes. A few years ago, the first efforts had been made to sequence and analyze the genomes of the first few lophotrochozoans, providing the very first glimpse into the genomic diversity of the superphylum. Available lophotrochozoan genomes so far include the conventionally (Sanger dideoxy reads) sequenced limpet *Lottia gigantea*, the annelids *Capitella teleta* (previously referred to as *Capitella capitata*) and *Helobdella robusta*, the parasitic and free-living flatworms *Schistosoma mansoni* [12] and *Schmidtea mediterranea* [13], as well as the Illumina/454-based genomes of the pacific oyster *Pinctada fucata* [14] and *Crassostrea gigas* [15], and, more recently, the annelid *P. dumerilii* (discussed later). In addition to these eight species, several other projects are underway.

Genome sizes vary significantly between lophotrochozoan species [16]: from 120 Mbp (in a flatworm *Olisthanella truncula* [17]) up to 7 Gbp (in a polychaete *Nephtys* [18]) (Table 1). Only two published lophotrochozoan repeat analyses are available: including that of the 1.2 Gbp *Pinctada fucata* [14] which indicates (potentially due to assembly quality) a relatively low repeat content of about 10%, and that of 637 Mbp *Crassostrea gigas* genome which is estimated to contain ~30% repeats [15]. It is still unclear whether the observed genome size variation among the lophotrochozoans is due to specific repeat expansions or other mechanisms such as whole genome duplications. Despite the high variability in genome sizes, previous reports, albeit limited to only a few loci (e.g. ParaHox and Antennapedia–class genes in *Platynereis* [19] and Hox genes in *Capitella* [20, 21], and Wnt [22] and Forkhead [23] in other

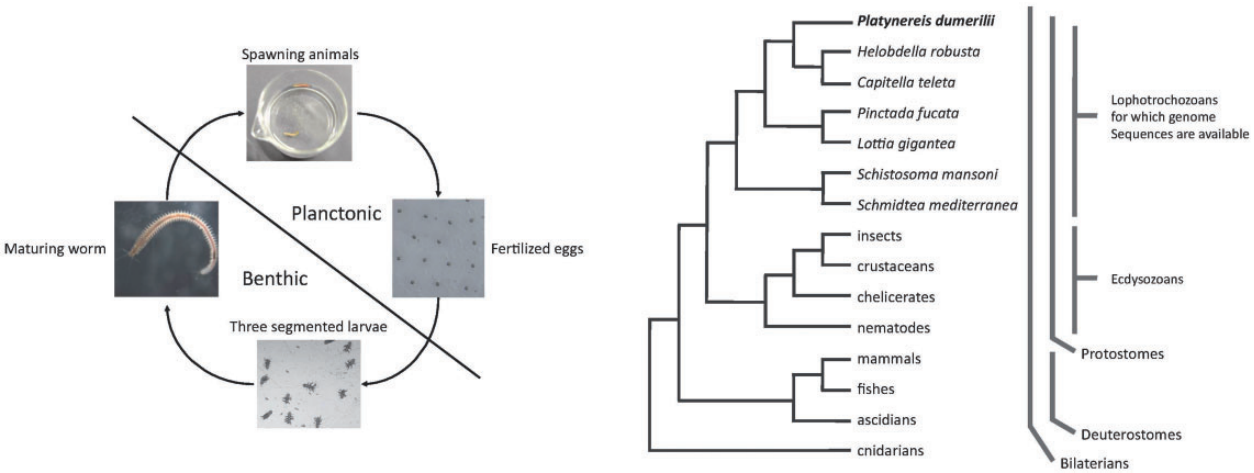

Figure 1: *Platynereis* life cycle and phylogenetic position. (**A**) Biphasic life cycle of *Platynereis*. Benthic transition occurs at around 5 dpf and commits the animal to a specific environment. For an in-depth description of the life cycle, see [8]. (**B**) Schematic bilaterian phylogeny depicting *P. dumerilii* in relation to other Lophotrochozoans for which genomic information is available (annelid branching according to [2]).

Table 1: Current state of the sequenced lophotrochozoan genomes and the available methods

	Platynereis dumerilii	*Capitella teleta*	*Helobdella robusta*	*Lottia gigantea*	*Pinctada fucata*	*Crassostrea gigas*	*Schmidtea mediterranea*	*Schistosoma mansonii*
Genomic								
Chromosomes (2*n*)	28 [38]	20 [39]	18 [40]	N.A.	28 [41]	20 [42]	8 [43]	16 [44]
Genome	Illumina/454	Sanger	Sanger	Sanger	Illumina/454	Illumina/454	Sanger	Sanger
EST	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Genome size	1 Gbp	324 Mbp	228 Mbp	348 Mbp	1.2 Gbp	637 Mbp	875 Mbp	370 Mbp
Developmental								
Expression fingerprint profiling	Yes [32]	No[a]	No[a]	No	No	No	No	No
Lineage/fate maps	Yes [8, 45, 46]	Yes [47]	Yes [48, 49]	No	No	No	No	No
Morpholinos RNAi	Morpholino	N.A.	Morpholino [50]	No	No	RNAi [51]	RNAi [52]	RNAi [53]
Transgenics	Transient	No	No	No	No	No	No	Yes [54]
Population studies								
Ecology studies	Yes; marine	Yes; marine	Yes; freshwater	Yes; marine	Yes; marine	Yes; marine	Yes; freshwater	Yes; parasitic
Genotyping data	Available	Available	Available	Available	Available	Available	Available	Available
Maintenance								
Lab facilities	Yes	Yes	Yes	N/A	N/A	N/A	Yes	Yes
Life cycle in the lab	3 months	1 month	2 months	N/A	N/A	N/A	1–2 weeks[b]	1 month

[a]Large-scale profiling methods have not been published yet but are possible due to stereotypic development.
[b]Asexual mode of reproduction.

lophotrochozoans), seem to suggest a higher degree of conservation of synteny (gene linkage) between lophotrochozoans and deuterostomes as compared with ecdysozoans and deuterostomes. However, no large-scale studies have been published so far, and thus the dynamics of lophotrochozoan linkage evolution are yet to be uncovered.

Other genomic characters such as intron splice site and indel position conservation, and other less frequently occurring events ('rare genomic changes') have been proposed as reliable characters for phylogeny inference and studies on the evolution of individual gene families [24, 25]. So far only one study exists that shows a high degree of intron splice site conservation in lophotrochozoans (based on *Platynereis* [26]), compared with most of the sequenced ecdysozoans. Additional support for high degree of conservation of genomic features comes also from non-coding elements, as has been revealed by a recent study that showed a high degree of miRNA repertoire conservation between *Platynereis* and key deuterostomes [27].

PLATYNEREIS: A LOPHOTROCHOZOAN MODEL SYSTEM TO STUDY MACRO-EVOLUTION

Not all lophotrochozoan species that have had their genome sequenced are well suited for long-distance evolutionary comparisons and at the same time are well suited for molecular studies (Table 1). *Platynereis* has proven to be one of the most accessible model systems for both genomic and experimental approaches in recent years. It has been kept in lab cultures since the 1950s [28] and has already been used in studies ranging from cell type and body plan evolution [29–35], to evolution of genomic characters such as introns [26] and microRNAs [27], to the studies of the evolution of plankton swimming behavior [36, 37].

The main advantages that make it a convenient species for use in the laboratory include mass spawning, transparency of embryos, synchronous and stereotypical development [8], little space requirements and the ease of maintenance and culture (Table 1). Despite being a relatively new model system for molecular biology, both descriptive (e.g. whole mount *in situ* expression profiling [55], high-throughput single cell expression analysis [32], reflection light sheet confocal microscopy, as described in [56]) and functional methods (such as morpholino knockdown, transgenesis) have been established. Those methods have successfully been combined to yield a complete single-cell level description of developing larvae, as has been shown by [32].

Platynereis genome size is estimated to be ~1 Gbp [57], comparable with the oyster *Pinctada fucata* (1.2 Gbp). Very little is known about the variation in

genome sizes between *Platynereis* and its sibling species, e.g. *P. bicanaliculata*, and other nereidids (such as *Nereis sp.*). Several studies [18] hint at high diversity in genome sizes within this group, varying by a factor of two, e.g. in *Alitta succinea* (previously named *Nereis succinea* which has an estimated genome size of 2.1 Gbp).

The sequencing of *Platynereis* genome is conducted as a community effort involving many laboratories worldwide using a combination of 454 and Illumina sequencing technologies from several individuals of an inbred strain. To counteract the difficulties arising from assembling a large genome, we apply a mixed insert size Illumina strategy [58], which also provides enough coverage to detect persistent polymorphisms and indel variation. To aid in future annotation of the genome, several transcriptome datasets have also been generated which cover different tissues and stages.

PLATYNEREIS: A MODEL SYSTEM FOR POPULATION MICRO-EVOLUTIONARY STUDIES

Polychaetes, and nereidids in particular, inhabit many ecological niches ranging from sandy sediment to coral reefs [9] (Figure 2). Despite such high environmental diversity, the individual species seem to implement the same strategies of adaptation (tube building, etc.) [60] and can readily adapt to new environments and/or switch to a different diet, one of the main reasons why *Platynereis* is easy to culture in the lab [28].

Over the past decades, *Platynereis sp.* has been a particular good system in classical population studies, in both pollution [61, 62] and ecological [63, 64] areas. Supported by the Association of European Marine Biological Laboratories (ASSEMBLE) [65] and Tara Oceans [66], *Platynereis* genome and genetic diversity is now being assessed across numerous locations, covering many different ecological niches from sandy sediment to algal beds and corals.

Previous morphological studies already identified high levels of variation between closely related *Platynereis* species [67] and within a single population [68]. Other reports [69, 70] also predict that genomic variability (e.g. genome size and ribosomal gene complement) and intra-specific variation are high as well. Not surprisingly, cryptic species have been identified in the clade [71]. Being a broadcast spawner, high polymorphism rates are expected for *Platynereis*, but little is known about the significance of these in a population context.

TOWARD COMBINED MICRO- AND MACRO-EVOLUTION CELLULAR-RESOLUTION MODELS

To begin analyzing macro- and micro-evolution in one system, one has to develop a model capable of providing and integrating such information. Taking advantage of the *Platynereis* technical toolbox, current work focuses on designing a cellular model that integrates variation at gene (allelic diversity, expression), cellular (expression profiling, cellular morphology and physiology) and ecological levels. This interplay is illustrated in Figure 3.

With regard to gene level variation within and between populations, both allelic composition and expression levels can be determined from next-generation (Illumina/454) sequencing data, although results have to be interpreted with caution for lowly expressed genes [72]. Although the majority of detected polymorphisms constitute single nucleotide polymorphisms (SNPs), which can be classified as being either neutral (synonymous) or amino acid changing (non-synonymous, Figure 3A, upper panel), other detectable variations include insertion/deletions (indels) of up to several hundred amino acids (Figure 3A, upper panel, gap regions) or even variation in domain order of a gene. Although it is hard to detect alleles with only a few SNPs using currently available expression profiling methods, it is possible to distinguish between the different structural variants using standard *in situ* protocols.

As to cellular variation, availability and easy-to-collect natural population of *Platynereis* allow high-throughput *in situ* screening to identify cell types expressing genes of interest in many individuals (Figure 3B, upper panel). This can be achieved by recently developed methods of single cell expression profiling [32, 37, 73], which allow the alignment of 3D confocal images of individual *in situ* experiments to an averaged model of larvae at a given developmental stage (using axonal scaffold or nuclei as landmarks). Additionally, this analysis can be supplemented with total transcriptome analysis of isolated single cells or tissues. Ultimately, this dataset does not only provide positional information, but can also be used to infer variation in the expression domain between individuals or populations.

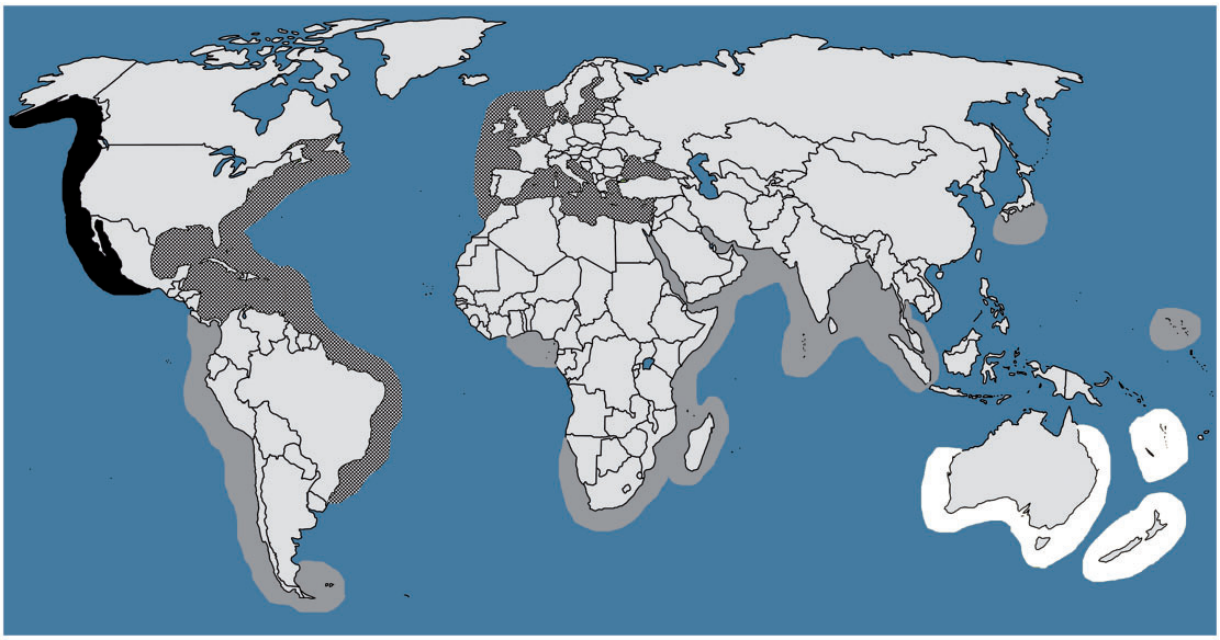

Figure 2: Known *Platynereis* sp. distribution (gray area). Numbers indicate estimated (corrected) synonymous substitution distances between populations. Dotted: Predominant *P. dumerilii* and related species. Black: predominantly *P. bicanaliculata*; white: predominantly *P. australis*. *Platynereis* coordinates from personal collections and OBIS [59].

Both genetic (i.e. allelic) and expression variation play a major role in defining the species in its natural environment and affect its diversification. Transcriptomic data from different species reveals macro-evolutionary trends such as evolutionary rates of gene family evolution, domain shuffling, expression differences, etc. In addition to the genetic data, the information on oceanographic (pH, salinity, etc.) and trophic (substrate and metabolomic) parameters of the system is also available (Figure 3C). With the established molecular techniques in *Platynereis,* it is possible to perturb candidate genes and assess the changes in development in the environmental context (e.g. pH, salinity tolerance, food preference, changes to life cycle, etc.).

Taken together, these data can be superimposed onto a three-dimensional cellular model of the animal (Figure 3D) with each cell assigned a certain morphology and expression fingerprint, accompanied by the variation in the expression, morphology or genetic polymorphisms (micro-evolution) and gene family evolutionary dynamics or even linkage (macro-evolution). Such a multi-scale cellular model can be used as a powerful hypothesis testing ground in evolutionary biology, as exemplified in the next paragraph.

USING MACRO/MICRO EVOLUTIONARY MODELS TO ELUCIDATE CELL TYPE EVOLUTION

Inferences about metazoan cell type evolution are based on parsimony arguments using comparative molecular and morphological 'fingerprints' (e.g. gene expression or cellular morphology; [33, 74]). Such fingerprints are informative about the evolutionary relationships between cell types. Those with most similar fingerprints are often considered 'sister cell types' that evolved from one and the same precursor by diversification (Figure 4) [75]. However, the fingerprint data may not be fully conclusive about the nature of the common ancestor cell and the evolutionary mechanisms behind its diversification. For example, if a given function is present in one cell type but not in its sister, it may be difficult to decide whether this is due to loss (i.e. subfunctionalization) or *de novo* acquisition of this function (i.e. neofunctionalization).

We now propose to complement the standard evo–devo fingerprint-based studies with micro-evolutionary variation data to be able to quantitatively assign each cellular function an evolutionary rate, i.e. to identify cell functions that appear to be

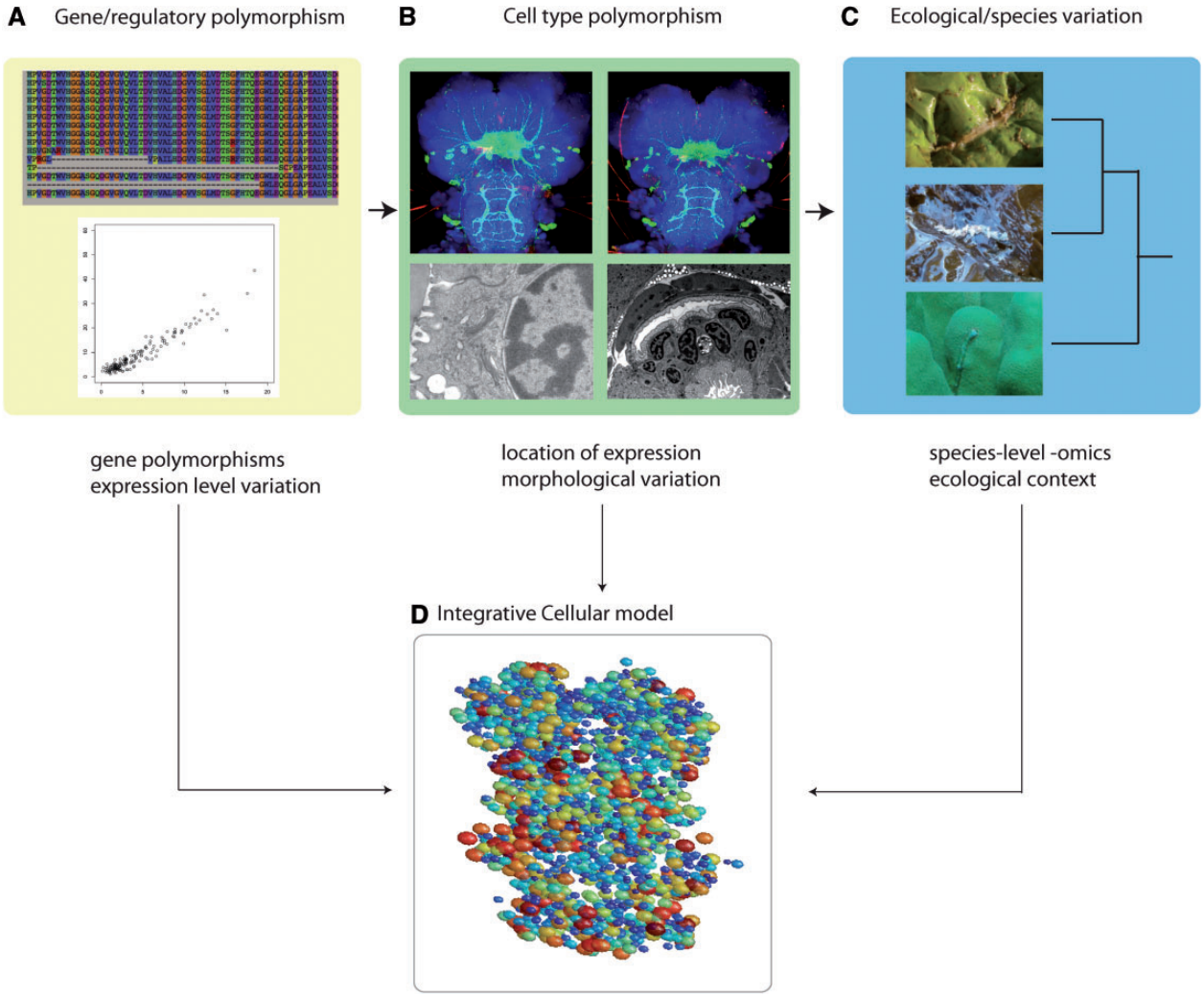

Figure 3: Resource integration in *Platynereis* eco–evo–devo studies. (**A**) Population transcriptomics revealing the allelic pool and expression variation; (**B**) *in situ* screen to identify cells expressing genes of interest and morphology; (**C**) comparative –omics analysis of closely and more distantly related species; (**D**) cellular models and data reconciliation. (A) *Top*: example of non-synonymous polymorphisms and indels within a *Platynereis* population; *bottom*: expression level polymorphisms identified on lane-to-lane plots (outliers from the diagonal). (B) *Top*: molecular fingerprint definition (variation in expression of the same gene) blue channel: DAPI nuclei stain; green: acetylated tubulin antibody; red: NBT/BCIP reflection for the gene of interest, chaetal staining is an artifact of reflection; *bottom*: morphological definition (using transmission electron microscopy). (C) *Platynereis* species inhabiting two different kelp species and a coral reef, phylogenetic relationships are indicated; (D) combining all available information (i.e. A, B, and C) into one cellular model using image registration, each sphere represents a single cell/nucleus, color and size indicate morphological variation among individuals (and can be extended to variation between species). Each nucleus is also associated with an expression and morphological fingerprint.

slow- (archaeofunctions) and fast-evolving (neofunctions). For our selection of marker genes for a given cell type, we can assess how variable they are in the natural environment: e.g. average duplication rates, sequence variation, selective pressure, as well as variations in the expression domain among and within natural populations at the cellular level. The more specific a gene is for a given cell type, the more likely it is that there is a direct relationship between genetic variation and phenotypic variation.

By computing an average of those variation parameters among the marker genes, we can assign a 'dispersal' rate of a cell type and its characteristic functions in its genetic space. We can then identify cell types that are more or less prone to genetic variation than others. Also, we can identify marker

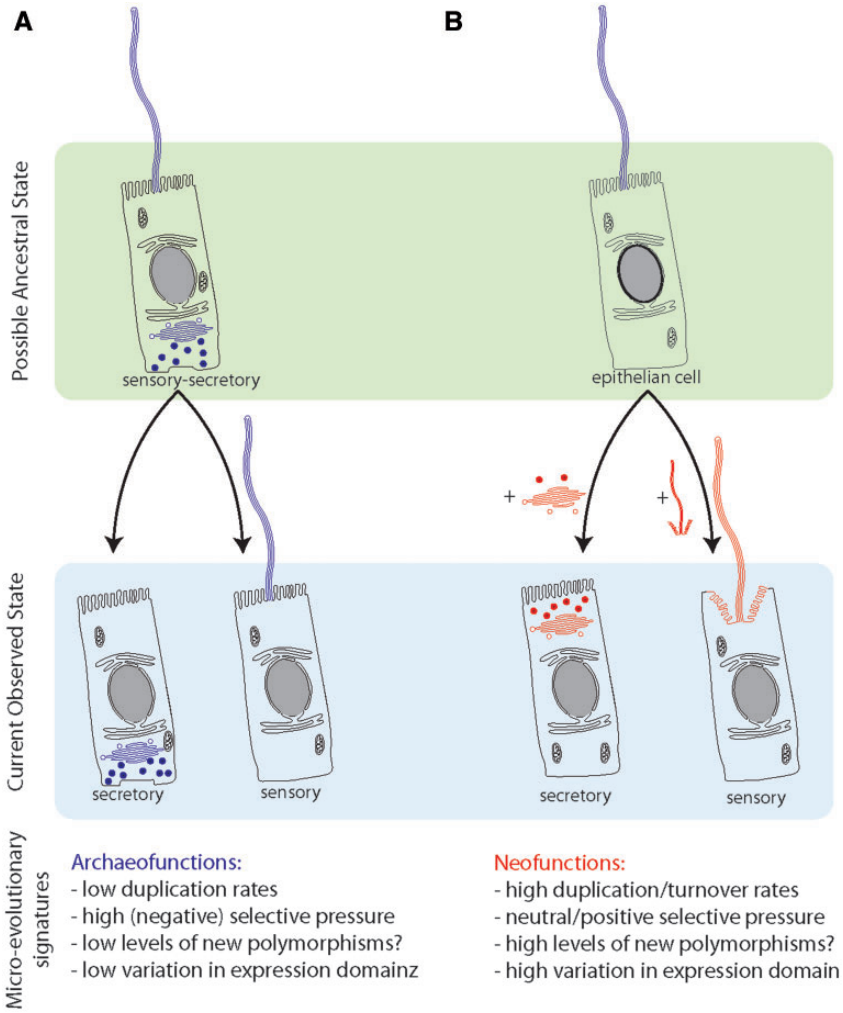

Figure 4: Implementation of micro-evolutionary data to help to distinguish between different evo–devo scenarios of cell type evolution. (**A**) sub-functionalization type of cell type evolution and (**B**) neofunctionalization. See text for more details.

genes, which are better suited for evo–devo inferences (e.g. having purifying selection and low copy number variation), and which are less reliable (e.g. fast evolving genes with variable expression domain and copy number).

To illustrate this, we give an example of sub- (Figure 4A) and neofunctionalization (Figure 4B) scenarios of cell type evolution [75]. For the sub-functionalization scenario (Figure 4A), because the functionality (archaeofunction) was already present in the ancestral cell type and maintained due to purifying selection, we expect to see a limited amount of accumulated changes. We would also expect to see little amount of recently duplicated genes, because (if kept) they are often associated with relaxed selective pressures and evolution of novel functions [76, 77]. Additionally, we might observe ancient and actively maintained paralogs (maintained allelic variants, as in [78]). If, on the other hand, the cell types with similar molecular fingerprint derived from an undifferentiated ancestral cell type (scenario in Figure 4B), we can suggest that the evolution of novel functionality (neofunctions) might have required higher rates of sequence and regulatory evolution: due to no or presence of modifying selection resulting in many amino acid substitutions in expressed genes, high levels of new polymorphisms, variation of expression domains, etc. We might also observe multiple (recent) paralogs indicating an ongoing evolutionary adaptation.

As yet, it is unclear whether cell type evolution is driven by high rates of sequence substitutions or just by few changes in the gene sequence [79] and/or regulatory landscape (as reported for sea urchin and

sea star [80]). To find out, comparative studies on cell type molecular fingerprints will have to be extended to genomic (and not just transcriptomic) level. Only then, we will be able to fully understand the underlying molecular mechanisms of cell type diversification.

CONCLUSIONS

Inferences about early bilaterian evolution so far were mainly based on molecular data from traditional model species (predominantly vertebrates, insects and nematodes). Current advances in the molecular analyses of the lophotrochozoans have changed our understanding of early bilaterian evolution. Because of the recent and still ongoing development of a combination of molecular and genomic tools, *Platynereis* stands out as one of the more accessible marine model organisms to address these questions and study evolution and development on both macro- and micro-evolutionary scales.

Key Points

- Understanding bilaterian cell type evolution requires models of both macro- and micro-evolution.
- Lophotrochozoans (and in particular *Platynereis*) are well positioned for such studies due to both pronounced retention of bilaterian ancestral characters and the presence of highly polymorphic, segregating natural strains.
- Recently published studies have established *Platynereis* as one of the main models in macro-evolutionary studies (comparative gene expression and cell type morphology).
- *Platynereis* is an emerging model system for population-level micro-evolutionary studies through the ongoing population – omics projects.
- Taking advantage of high-resolution expression profiling in *Platynereis*, it is possible to combine both macro- and micro-evolutionary data into a single cell type model of the organism and use it for evolutionary hypothesis testing.

Acknowledgements

O.S. is supported by a PhD fellowship from Boehringer Ingelheim Fonds. T.L. is supported by an EIPOD fellowship from EMBL. Platynereis sampling is supported by ASSEMBLE grant to O.S. The authors wish to thank David Weisblat, Mette Handberg-Thorsager and Pavel Vopalensky for commenting on the manuscript.

References

1. Stearn CW, Carroll RL. *Paleontology: The Record of Life*. New York: John Wiley, 1989.
2. Struck TH, Paul C, Hill N, *et al*. Phylogenomic analyses unravel annelid evolution. *Nature* 2011;**471**:95–8.
3. Gould SJ. *Wonderful Life: The Burgess Shale and the Nature of History*. New York: W.W. Norton, 1989.
4. Peterson KJ, Cotton JA, Gehling JG, *et al*. The Ediacaran emergence of bilaterians: congruence between the genetic and the geological fossil records. *Philos Trans R Soc Lond B Biol Sci* 2008;**363**:1435–43.
5. Valentine JW. Late precambrian bilaterians: grades and clades. *Proc Natl Acad Sci U S A* 1994;**91**:6751–7.
6. Halanych KM, Bacheller JD, Aguinaldo AM, *et al*. Evidence from 18S ribosomal DNA that the lophophorates are protostome animals. *Science* 1995;**267**:1641–3.
7. Aguinaldo AM, Turbeville JM, Linford LS, *et al*. Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature* 1997;**387**:489–93.
8. Fischer AH, Henrich T, Arendt D. The normal development of *Platynereis dumerilii* (Nereididae, Annelida). *Front Zool* 2010;**7**:31.
9. Rouse GW, Pleijel F. *Polychaetes*. Oxford; New York: Oxford University Press, 2001.
10. Foster N. FRESHWATER POLYCHAETES (ANNELIDA) OF NORTH AMERICA. *Water Pollution Control Research Series*. U.S. Environemntal Protection agency, 1972.
11. Giribet G. Assembling the lophotrochozoan (=spiralian) tree of life. *Philos Trans R Soc Lond B Biol Sci* 2008;**363**:1513–22.
12. Berriman M, Haas BJ, LoVerde PT, *et al*. The genome of the blood fluke *Schistosoma mansoni*. *Nature* 2009;**460**:352–8.
13. Robb SM, Ross E, Sanchez Alvarado A. SmedGD: the *Schmidtea mediterranea* genome database. *Nucleic Acids Res* 2008;**36**:D599–606.
14. Takeuchi T, Kawashima T, Koyanagi R, *et al*. Draft genome of the pearl oyster *Pinctada fucata*: a platform for understanding bivalve biology. *DNA Res* 2012;**19**:117–30.
15. Zhang G, Fang X, Guo X, *et al*. The oyster genome reveals stress adaptation and complexity of shell formation. *Nature* 2012;**490**:49–54.
16. Gregory TR. *Animal Genome Size Database*. http://www.genomesize.com, Last accessed October 2nd, 2012.
17. Gregory TR, Hebert PD, Kolasa J. Evolutionary implications of the relationship between genome size and body size in flatworms and copepods. *Heredity (Edinb)* 2000;**84**(Pt 2):201–8.
18. Conner WG, Hinegardner R, Bachmann K. Nuclear DNA amounts in polychaete annelids. *Cell Mol Life Sci* 1972;**28**:1502–4.
19. Hui JH, McDougall C, Monteiro AS, *et al*. Extensive chordate and annelid macrosynteny reveals ancestral homeobox gene organization. *Mol Biol Evol* 2012;**29**:157–65.
20. Hui JH, Raible F, Korchagina N, *et al*. Features of the ancestral bilaterian inferred from *Platynereis dumerilii* ParaHox genes. *BMC Biol* 2009;**7**:43.
21. Frobius AC, Matus DQ, Seaver EC. Genomic organization and expression demonstrate spatial and temporal Hox gene colinearity in the lophotrochozoan Capitella sp. I. *PLoS One* 2008;**3**:e4004.
22. Cho SJ, Valles Y, Giani VC, Jr, *et al*. Evolutionary dynamics of the wnt gene family: a lophotrochozoan perspective. *Mol Biol Evol* 2010;**27**:1645–58.

23. Shimeld SM, Boyle MJ, Brunet T, *et al*. Clustered Fox genes in lophotrochozoans and the evolution of the bilaterian Fox gene cluster. *Dev Biol* 2010;**340**:234–48.
24. Putnam NH, Srivastava M, Hellsten U, *et al*. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science* 2007;**317**:86–94.
25. Rokas A, Holland PW. Rare genomic changes as a tool for phylogenetics. *Trends Ecol Evol* 2000;**15**:454–9.
26. Raible F, Tessmar-Raible K, Osoegawa K, *et al*. Vertebrate-type intron-rich genes in the marine annelid *Platynereis dumerilii*. *Science* 2005;**310**:1325–6.
27. Christodoulou F, Raible F, Tomer R, *et al*. Ancient animal microRNAs and the evolution of tissue identity. *Nature* 2010;**463**:1084–8.
28. Fischer A, Dorresteijn A. The polychaete *Platynereis dumerilii* (Annelida): a laboratory animal with spiralian cleavage, lifelong segment proliferation and a mixed benthic/pelagic life cycle. *Bioessays* 2004;**26**:314–25.
29. Denes AS, Jekely G, Steinmetz PR, *et al*. Molecular architecture of annelid nerve cord supports common origin of nervous system centralization in bilateria. *Cell* 2007;**129**: 277–88.
30. Arendt D, Technau U, Wittbrodt J. Evolution of the bilaterian larval foregut. *Nature* 2001;**409**:81–5.
31. Steinmetz PR, Kostyuchenko RP, Fischer A, *et al*. The segmental pattern of otx, gbx, and Hox genes in the annelid *Platynereis dumerilii*. *Evol Dev* 2011;**13**:72–9.
32. Tomer R, Denes AS, Tessmar-Raible K, *et al*. Profiling by image registration reveals common origin of annelid mushroom bodies and vertebrate pallium. *Cell* 2010;**142**:800–9.
33. Tessmar-Raible K, Raible F, Christodoulou F, *et al*. Conserved sensory-neurosecretory cell types in annelid and fish forebrain: insights into hypothalamus evolution. *Cell* 2007;**129**:1389–1400.
34. Arendt D, Tessmar-Raible K, Snyman H, *et al*. Ciliary photoreceptors with a vertebrate-type opsin in an invertebrate brain. *Science* 2004;**306**:869–71.
35. Dray N, Tessmar-Raible K, Le Gouar M, *et al*. Hedgehog signaling regulates segment formation in the annelid *Platynereis*. *Science* 2010;**329**:339–42.
36. Jekely G, Colombelli J, Hausen H, *et al*. Mechanism of phototaxis in marine zooplankton. *Nature* 2008;**456**:395–9.
37. Conzelmann M, Offenburger SL, Asadulina A, *et al*. Neuropeptides regulate swimming depth of *Platynereis* larvae. *Proc Natl Acad Sci U S A* 2011;**108**:E1174–83.
38. Jha AN, Hutchinson TH, Mackay JM, *et al*. The chromosomes of *Platynereis dumerilii* (Polychaeta: Nereidae). *J Marine Biol Assoc UK* 1995;**75**:551–62.
39. Grassle JP, Gelfman CE, Mills SW. Karyotypes of Capitella sibling species, and of several species in the related genera Capitellides and Capitomastus (Polychaeta). *Bull Biol Soc Wash* 1987;**7**:77–88.
40. Cichocka J, Bielecki A. Biological diversity of leeches (Clitellata: Hirudinida) based on characteristics of the karyotype. *Wiad Parazytol* 2008;**54**:309–14.
41. Wada K. Number and gross morphology of chromosomes in the pearl oyster, *Pinctada fucata* (GOULD): collected from two regions of Japan. *Venus* 1976;**35**:9–14.
42. Thiriot-Quievreux C, Pogson GH, Zouros E. Genetics of growth rate variation in bivalves: aneuploidy and heterozygosity effects in a Crassostrea gigas family. *Genome* 1992;**35**:39–45.
43. Baguna J, Carranza S, Pala M, *et al*. From morphology and karyology to molecules. New methods for taxonomical identification of asexual populations of freshwater planarians. A tribute to Professor Mario Benazzi. *Italian J Zool* 1999;**66**:207–14.
44. Short RB, Grossman AI. Conventional giemsa and C-banded karyotypes of *Schistosoma mansoni* and *S. rodhaini*. *J Parasitol* 1981;**67**:661–71.
45. Ackermann C, Dorresteijn A, Fischer A. Clonal domains in postlarval *Platynereis dumerilii* (Annelida: Polychaeta). *J Morphol* 2005;**266**:258–80.
46. Dorresteijn AWC. Quantitative analysis of cellular differentiation during early embryogenesis of *Platynereis dumerilii*. *Roux Arch Dev Biol* 1990;**199**:14–30.
47. Meyer NP, Boyle MJ, Martindale MQ, *et al*. A comprehensive fate map by intracellular injection of identified blastomeres in the marine polychaete *Capitella teleta*. *Evodevo* 2010;**1**:8.
48. Zhang SO, Weisblat DA. Applications of mRNA injections for analyzing cell lineage and asymmetric cell divisions during segmentation in the leech *Helobdella robusta*. *Development* 2005;**132**:2103–13.
49. Gline SE, Kuo DH, Stolfi A, *et al*. High resolution cell lineage tracing reveals developmental variability in leech. *Dev Dyn* 2009;**238**:3139–51.
50. Song MH, Huang FZ, Chang GY, *et al*. Expression and function of an even-skipped homolog in the leech *Helobdella robusta*. *Development* 2002;**129**:3681–92.
51. Huvet A, Fleury E, Corporeau C, *et al*. In vivo RNA interference of a gonad-specific transforming growth factor-beta in the Pacific oyster *Crassostrea gigas*. *Mar Biotechnol (NY)* 2012;**14**:402–10.
52. Sanchez Alvarado A, Newmark PA. Double-stranded RNA specifically disrupts gene expression during planarian regeneration. *Proc Natl Acad Sci U S A* 1999;**96**:5049–54.
53. Stefanic S, Dvorak J, Horn M, *et al*. RNA interference in *Schistosoma mansoni* schistosomula: selectivity, sensitivity and operation for larger-scale screening. *PLoS Negl Trop Dis* 2010;**4**:e850.
54. Kines KJ, Morales ME, Mann VH, *et al*. Integration of reporter transgenes into *Schistosoma mansoni* chromosomes mediated by pseudotyped murine leukemia virus. *FASEB J* 2008;**22**:2936–48.
55. Jekely G, Arendt D. Cellular resolution expression profiling using confocal detection of NBT/BCIP precipitate by reflection microscopy. *Biotechniques* 2007;**42**:751–5.
56. Tomer R, Khairy K, Amat F, *et al*. Quantitative high-speed imaging of entire developing embryos with simultaneous multiview light-sheet microscopy. *Nat Methods* 2012;**9**: 755–63.
57. Soldi R, Ramella L, Gambi MC, *et al*. Genome size in polychaetes: relationship with body length and life habit. In: Dauvin J-C, *et al* (ed). *Actes de la 4ème Conférence internationale des Polychètes, Angers, France*. Vol. 162. Mémoires du Muséum national d'histoire naturelle. Série A, Zoologie, 1994, 129–35.
58. Li R, Fan W, Tian G, *et al*. The sequence and de novo assembly of the giant panda genome. *Nature* 2010;**463**: 311–7.

59. UNESCO IOCIo. *The Ocean Biogeographic Information System*. http://www.iobis.org.
60. Cram A, Evans SM. Stability and lability in the evolution of behaviour in nereid polychaetes. *Animal Behav* 1980;**28**: 483–90.
61. Bellan Gr. Relationship of pollution to rocky substratum polychaetes on the French Mediterranean coast. *Marine Pollution Bull* 1980;**11**:318–21.
62. Hutchinson TH, Jha AN, Dixon DR. The polychaete *Platynereis dumerilii* (Audouin and Milne-Edwards): a new species for assessing the hazardous potential of chemicals in the marine environment. *Ecotoxicol Environ Saf* 1995;**31**: 271–81.
63. Hardege JD. Nereidid polychaetes as model organisms for marine chemical ecology. *Hydrobiologia* 1999;**402**:145–61.
64. Jacobs RPWM, Pierson ES. Zostera marina spathes as a habitat for *Platynereis dumerilii* (Audouin and Milne-Edwards, 1834). *Aquat Bot* 1979;**6**:403–6.
65. ASSEMBLE. *Association of European Marine Biological Laboratories*. http://www.assemblemarine.org.
66. Karsenti E, Acinas SG, Bork P, *et al*. A holistic approach to marine eco-systems biology. *PLoS Biol* 2011;**9**:e1001177.
67. Schneider S, Fischer A, Dorresteijn AWC. A morphometric comparison of dissimilar early development in sibling species of *Platynereis* (Annelida, Polychaeta). *Roux Arch Dev Biol* 1992;**201**:243–56.
68. Rhode B. Development and differentiation of the eye in *Platynereis dumerilii* (Annelida, Polychaeta). *J Morphol* 1992; **212**:71–85.
69. Gambi MC, Ramella L, Sella G, *et al*. Variation in genome size in benthic polychaetes: systematic and ecological relationships. *J Marine Biol Assoc UK* 1997;**77**:1045–57.
70. Hui JHL, Kortchagina N, Arendt D, *et al*. Duplication of the ribosomal gene cluster in the marine polychaete *Platynereis dumerilii* correlates with ITS polymorphism. *J Marine Biol Assoc UK* 2007;**87**:443–9.
71. Weinberg JR, Starczak VR, Mueller C, *et al*. Divergence between populations of a monogamous polychaete with male parental care: premating isolation and chromosome variation. *Marine Biol* 1990;**107**:205–13.
72. McIntyre LM, Lopiano KK, Morse AM, *et al*. RNA-seq: technical variability and sampling. *BMC Genomics* 2011;**12**: 293.
73. OLiGO. *A Tool for Automated Confocal Registration and Expression Profiling*. http://4dx.embl.de/arendt/oligo/.
74. Arendt D. The evolution of cell types in animals: emerging principles from molecular studies. *Nat Rev Genet* 2008;**9**: 868–82.
75. Arendt D, Hausen H, Purschke G. The 'division of labour' model of eye evolution. *Philos Trans R Soc Lond B Biol Sci* 2009;**364**:2809–17.
76. Ohta T. Evolution by gene duplication and compensatory advantageous mutations. *Genetics* 1988;**120**:841–7.
77. Ohta T. Role of gene duplication in evolution. *Genome* 1989;**31**:304–10.
78. Jones FC, Grabherr MG, Chan YF, *et al*. The genomic basis of adaptive evolution in threespine sticklebacks. *Nature* 2012;**484**:55–61.
79. Lynch VJ, Tanzer A, Wang Y, *et al*. Adaptive changes in the transcription factor HoxA-11 are essential for the evolution of pregnancy in mammals. *Proc Natl Acad Sci U S A* 2008;**105**: 14928–33.
80. Hinman VF, Nguyen A, Davidson EH. Caught in the evolutionary act: precise cis-regulatory basis of difference in the organization of gene networks of sea stars and sea urchins. *Dev Biol* 2007;**312**:584–95.