Gray anatomy: phylogenetic patterns of somatic gonad structures and reproductive strategies across the Bilateria

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Synopsis The last common ancestor of extant bilaterian animals is often referred to as "Urbilateria". Comparative studies of development in a variety of laboratory animals, both traditional model systems and newer "emerging" models, have resulted in many proposals as to the morphological and developmental genetic characteristics of Urbilateria. Most of these proposals are concerned with the development and emergence of external morphology, such as appendages, eyes, and ectodermal segmentation. Less attention has been paid to the evolutionary developmental biology of organogenesis. Arguably, one of the most important aspects of urbilaterian organogenesis would have been gonadogenesis, since Urbilateria must have successfully generated gametes and developed a strategy for extrusion and fertilization, in order to be the ancestor of all living Bilateria. This article considers what is known about gonadogenesis and reproductive strategies in extant metazoans, and searches for phylogenetic patterns that suggest what shared characteristics of these processes Urbilateria might have displayed. I conclude that the data presently available cannot suggest homologies of the somatic components of metazoan gonads, and that convergent evolution has resulted in many different morphological, and possibly molecular genetic, solutions to the various problems posed by sexual reproduction.

Introduction

> Evo-devologists are intensely fascinated with ancestors. (Jenner 2006, p 387)

Many practitioners of evolutionary developmental biology would feel comfortable discussing some aspects of the external morphology of the last common ancestor of bilaterian animals. The comparative developmental genetics of, for example, appendages (Panganiban et al. 1997; Minelli 2003), segments (Balavoine and Adoutte 2003; Peel and Akam 2003), and photoreceptors (Kozmik et al. 2003; Gehring 2005; Kozmik 2005), have been studied in a wide enough range of extant species to allow many researchers to begin to suggest ground plans for these developmental characters. However, leaving aside discussions of the origin of mesoderm (Technau and Scholz 2003; Martindale et al. 2004), and with the notable exception of the development of the heart (Bodmer and Venkatesh 1998), there are few proposals for the development and evolution of urbilaterian internal organs.

The internal reproductive system of any sexually reproducing animal clearly deserves the attention of developmental biologists, and that of Urbilateria even more so. As hypothetical a construct as Urbilateria is, if it did, indeed, give rise to today's diverse animal lineages, its reproductive system must have been functioning rather well. In this article, I present an overview of the available comparative data on the somatic aspects of reproduction, with the aim of identifying emerging general patterns that suggest both how Urbilaterian reproduction might have been achieved, and how putative Urbilaterian somatic gonads could have been modified with descent to result in the diversity of extant reproductive systems.

Before proceeding, I will first clarify my expectations as to what kind of information the study of extant species can give us about Urbilaterian morphology. What most evo-devo researchers usually mean when they speak of Urbilateria, is that it was the earliest animal whose body plan comprised those characters shared by protostomes and deuterostomes, as revealed by studies of extant members of both clades (Carroll et al. 2005). Arendt (2005) neatly sums up this widespread, zootype-based (Slack et al. 1993) assumption when

he suggests that we can understand, for example, last common ancestor (LCA) gene expression, by examining two different extant descendant species in the following way: "If, in two distinct species, orthologous transcription factors are expressed in a sufficiently similar and specific manner, these expression regions are considered homologous . . . and should have been present in the body plan of their last common ancestor." It is worth noting, however, that the founding ancestors of the clades containing each species, had already diverged from the LCA by at least a species-level distance (hence their definition as distinct from the LCA), and that the LCA of the two species thus cannot automatically be assumed to have body plans, or any other characteristic, identical to those of the extant species (Jenner 2006; Valentine 2006). In this article, therefore, I wish to avoid suggesting that extant reproductive systems are simply variations on a defined metazoan reproductive "zootype" or "*Bauplan*". A great weakness of the current evo-devo approach stems from dilution of explanatory force with inappropriate fixations on strict, confining definitions of this kind (Scholtz 2004, 2005; Hübner 2005). I will briefly review the current and historical literature on somatic gonad anatomy, embryonic specification and development, studies obviously all carried out on extant species, but will not infer from these data that Urbilateria must have had specific, archetypical genetic or developmental characteristics either of the anatomy of its reproductive system anatomy or its reproductive strategies. Rather, I will suggest that these data can tell us what kinds of general features, or basic pattern, its reproductive system was likely to have had, in order for it to have given rise to these systems as manifest in extant protostome and deuterostome lineages.

Having clarified that my mission is one of identifying general patterns, rather than of searching for archetypal definitions, we can ask what approaches are usually used to suggest the putative morphological and/or developmental genetic characteristics of Urbilateria. Comparative morphology and embryology, gene expression patterns, and functional genetic testing have all been helpful approaches in the study of external morphologies. As we will see, some of these same approaches, together with behavioral observations of reproductive strategy, have yielded data useful for considering gonad structure, gonad function, and gonadogenesis in the last common ancestor of bilaterians. Several questions about this aspect of Urbilateria come to mind: was it hermaphroditic, parthenogenetic, or did separate sexes exist? Did it have a discrete gonad?

If so, from which germ layer did it originate? How was fertilization achieved? In order to begin to examine some of these questions, we first need to define the components of functional reproductive systems.

Components of bilaterian reproductive systems

There is a minimum of two requirements for successful reproduction: (1) cells to make gametes, and (2) a fertilization strategy. Most bilaterian reproductive systems possess a third critical element: a dedicated group of somatic cells that enclose, support, and extrude the gametogenic cells.

Reproductive strategies

> Many hours of careful observation by several investigators, including myself, have failed to reveal any notable sexual behaviour on the part of kinorhynchs. It is assumed that copulation takes place, but the act has not been observed. (Higgins 1974, p 512)

When considering Urbilateria, we will of course be in the shoes of Higgins and other investigators of the elusive kinorhynchs, in that by definition some kind of reproductive strategy must have been employed, but we have no way of knowing what it was. Once gametes have been made, if fertilization is necessary, gametes of separate sexes must be brought together. Fertilization can be wholly external (gametes of both sexes are released without copulation), wholly internal (gametes of one sex are deposited within the individual of the opposite sex, via copulation; or, in the case of self-fertile hermaphrodites, gametes of both sexes are already contained within one individual), or external–internal (gametes of one sex are released without copulation, then taken up by the opposite sex, so that fertilization is internal). The type of reproductive strategy employed depends on the anatomy of the somatic reproductive system. For example, genital ducts and copulatory organs are prerequisites for wholly internal fertilization. For this reason, we will only be able to begin speculation about an Urbilaterian reproductive strategy, once we have identified some patterns of comparative metazoan structure of the somatic gonad.

The somatic gonad

> In the Malayan *P. epitocus* the oogonia . . . on reaching a certain size, begin to phagocytose the gut of the mother. They consume the gut, then the nephridia and the musculature, everything, until nothing is left of the last segments of the maternal

organism but a thin cuticle. That cuticle breaks open and liberates the ova. In the above instance the antagonism between the genital products and the maternal organism takes such a violent course because of the absence of special adaptations for discharging them to the exterior... Most animals have taken another path and created adaptations that provide for removal of genital products from the organism. (Beklemishev 1964)

For many humans, copulation is among the most pleasurable of experiences, and childbirth among the most painful. However, compared to the methods of gamete or zygote extrusion employed by many nonhuman metazoans, having a dedicated, flexible and nonobligate-lethal exit route is rather beneficial. What all somatic reproductive systems have in common is that they comprise a network of nongametogenic cells whose role is to support, enclose, and if necessary, transport and expel the gametic products of the individual.

Beklemishev (1964) defined five components of the somatic reproductive system as: (1) genital glands or gonads (where gametogenesis takes place); (2) genital ducts (used for storing, transporting, or extruding gametic products); (3) copulatory organs (used for transferring gametes between individuals of the opposite sex); (4) adaptations creating envelopes for ova; (5) adaptations for bearing live young. I will use these five categories to describe the reproductive systems of the metazoan phyla, and as will become evident, a successful reproductive strategy may involve all, or none, of these elements.

Comparative data on specification of the somatic gonad

Anatomical studies of members of most extant bilaterian phyla provide data on the structure of the somatic reproductive system. More difficult to obtain are data on the developmental origin of the system, and on its functioning during reproduction, as these depend on availability of reliably staged developmental intermediates, and direct observations of copulation and/or fertilization, respectively. What is immediately apparent even from the data available, however, is that on a bilaterian scale, a strictly phylogenetic consideration of the anatomy of the reproductive system makes no sense without also considering life history and environmental factors.

Figure 1 illustrates the complexity of the reproductive system across the metazoans according to the five categories proposed by Beklemishev (1964). These data, described briefly in the following section, were compiled from references cited by Exavour and Akam (2003) and from (Beklemishev 1964; Giese and Pearse 1974–1989; Brusca and Brusca 2003).

Within both protostomes and deuterostomes, the full range of complexities of reproductive systems is observed, from free-floating gametes within the body cavity that are extruded by epidermal rupture to engage in external fertilization, to gametes confined within elaborate gonads, that can only be exposed to gametes of the opposite sex through copulation, and travel through dedicated ducts to uteri specialized for viviparity.

Among the protostomes, the structure of the reproductive system can vary not only among phyla, but also within a single phylum. For example, within the Annelida, leeches have true gonads and gonoducts, as do oligochaetes and some polychaetes. Many polychaete species, however, lack discrete gonads. Instead, their gametes mature in coelomic cavities from free-floating gametogonia, are released by rupture of the body wall, and undergo external fertilization in the water column (Beklemishev 1964). Some Onychophorans have not only complex gonad structures, but also uteri: in viviparous species, fertilization is internal, embryos develop in uteri, and animals give birth to live young (Manton 1949; von Herzberg et al. 1980). As in many segmented protostomes, components of the somatic gonad are formed from mesodermal cells of the splanchnic dorsal coelomic wall (Manton 1949; Anderson 1973).

Among the deuterostomes, *Xenoturbella* has the simplest known reproductive system: as in many sponges, cnidarians, and flatworms, gametes develop freely in the coelom and are extruded through the mouth upon maturity (Beklemishev 1964). Many marine invertebrate deuterostomes have discrete gonads and gonoducts, but lack copulatory organs, with fertilization takes place in the water column. In echinoderms, for example, the gonads develop closely associated with the coelom, and have a germinal epithelium on the inner lining of the gonad from which gametogenic cells arise (Houk and Hinegardner 1980). Gonoducts lead from the gonads directly to the outside of the animal, as gametes of both sexes are released into the water column for fertilization. Mammals have, of course, developed specialized copulatory organs, as well as adaptations for internal fertilization, embryonic development, and viviparity. Although data on the layer from which the somatic gonad originates is lacking for many deuterostomes, at least in mammals, the somatic gonad probably derives from
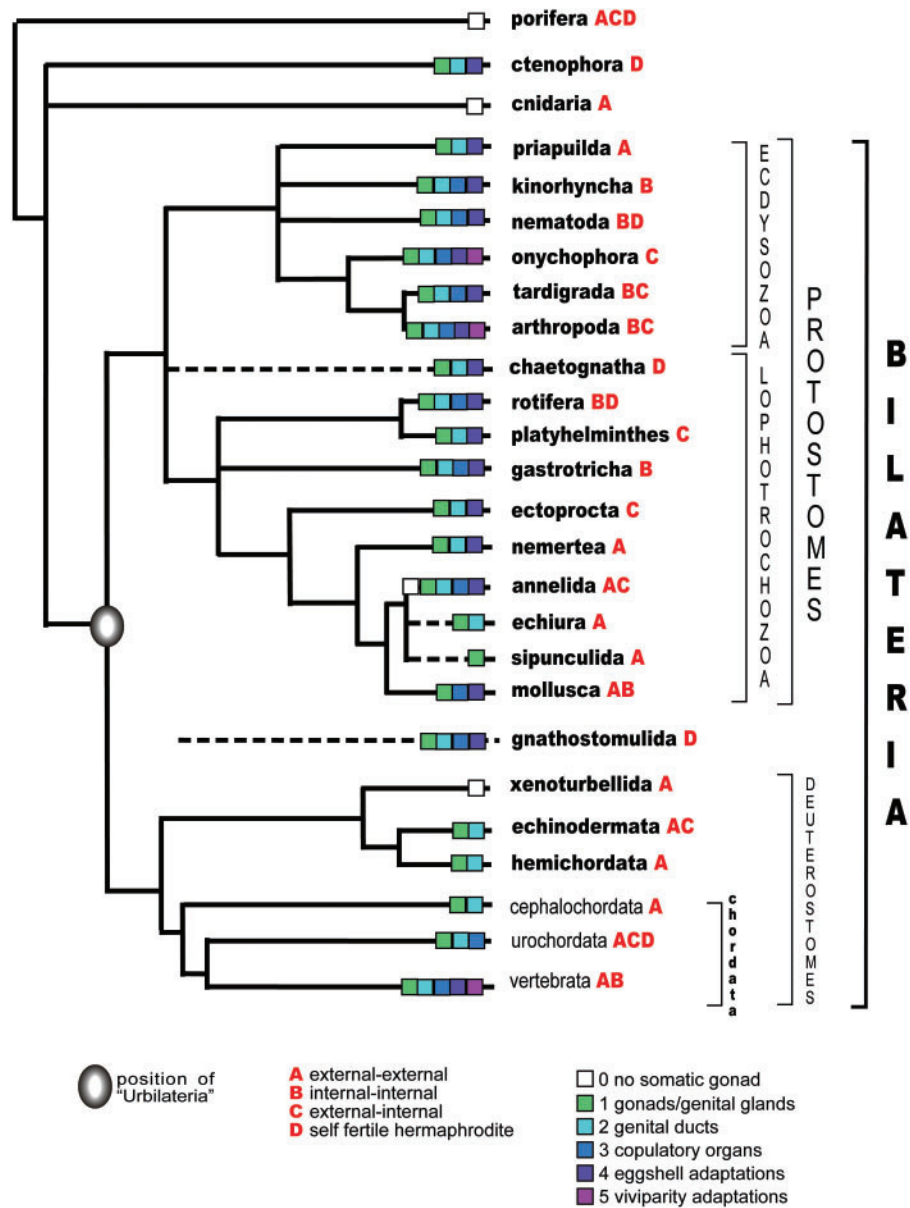
Fig. 1 Reproductive strategies and somatic reproductive systems across the metazoa. Possession by metazoan phyla of none (0) or some (1 through 5) of the five components of somatic reproductive systems are plotted on a metazoan phylogeny, as are the reproductive strategies employed. Red letters A through D indicate reproductive strategies employed: (**A**) external–external (e.g. gametes of both sexes released into a water column); (**B**) internal–internal (e.g. copulation); (**C**) external–internal (e.g. deposition and uptake of spermatophores); (**D**) self-fertile hermaphroditism. Color-coded numbers 0 through 5 indicate the following components: (0) white: no somatic gonad (1) green: gonads or genital glands (envelope of somatic cells enclosing gametogenic cells); (2) turquoise: genital ducts (leading from gonads to gonopores for extrusion of gametes); (3) blue: copulatory organs (may be used either for internal fertilization or external deposition of gametes, e.g. via spermatophores); (4) purple: adaptations for creating eggshells for ova (including vitelline membranes and chorions, but excluding extraembryonic membranes of zygotic origin); (5) magenta: adaptations for viviparity (including uteri and placentae). Italics indicate phyla containing members in more than one category. Data compiled from references cited by Exavour and Akam (2003) and from (Beklemishev 1964; Giese and Pearse 1974–1989; Brusca and Brusca 2003).

the mesonephros and the adjacent coelomic epithelium (McLaren 2000).

For many of the metazoans that have been studied, it is clear that the somatic and germinal components of the reproductive system are specified or "sequestered" separately during development, that

is, they share limited or no lineage. The huge diversity in somatic reproductive systems, contrasted with the relatively stable origin of primordial germ cells (Extavour and Akam 2003; Extavour 2007a, 2007b), should therefore not be surprising, given that independently sequestered lineages may display

modular independence in morphological evolution. West-Eberhard neatly summarized this by saying that "an increase in modularity...sometimes appears to have contributed to increased diversification of *that aspect of the phenotype* during the history of a taxon." [West-Eberhard 2003, p 87 (italics original)].

Somatic gonads in bilaterian outgroups

Examining characters of bilaterian outgroups (sponges, ctenophores, and cnidarians) sometimes helps to suggest a reasonable origin or developmental link to bilaterian features. In the case of the somatic gonad, however, these comparisons shed little light on the problem. Sponges lack true gonads, although some species do have "nutritive cells" associated with developing gametes. Although colonial cnidarians sometimes display shared "genital organs" in the form of gonophores (which may be reproductive zooids, polyps or buds), most cnidarians also lack true gonads, with gametogenesis taking place in intraepithelial spaces, or within mesoglea. Ctenophores are largely hermaphroditic, and gametes develop in endodermal organ structures that display canals for gamete expulsion. Some Acoels lack gonads, with gametogenic cells scattered in the parenchyma, but others display compact, paired, ovaries, and many have male copulatory organs.

Molecular mechanisms of somatic gonad specification

Data on the molecular mechanisms specifying the fate of the somatic gonad is limited to a few laboratory model organisms. In mice, embryonic gonads are derived from intermediate mesoderm, and first appear as paired thickenings of the coelomic epithelium, in close association with the mesonephros. By embryonic day 10, the embryonic gonads, together with primordial germ cells that have migrated to the location of the somatic gonad primordium, make up the genital ridges (reviewed by McLaren 2000; Cederroth et al. 2007). Gonadal differentiation then proceeds along the male or female pathway, largely as a result of hormone secretions produced by various types of somatic gonad cell, although there is some evidence that chromosomally-determined, cell-autonomous sex determination may also play a role (Kim and Capel 2006; Ottolenghi et al. 2007). Ovarian development relies on the activity of the Wnt4 signaling pathway, while testis development appears to proceed via *Sry*-induced Fgf9/Sox9 pathway activity (reviewed by Cederroth et al. 2007). In nematode worms, the somatic gonad derives from first cells of the larval

stage, i.e., Z1 and Z4 (Kimble and Hirsh 1979; Sulston et al. 1983). (Contrary to the relationship suggested by their nomenclature, these cells are not sister cells of the primordial germ cells Z2 and Z3). Z1 and Z4 are descendants of the third-cleavage mesodermal precursor cell MS, whose initial fate is the result both of segregation of cytoplasmic determinants during early cleavages, and of signaling interactions between early blastomeres (reviewed by Maduro 2006). Further somatic gonadal differentiation involves signaling pathways, including the Notch and Wnt pathways (Seydoux and Greenwald 1989; Hubbard and Greenstein 2000; Chang et al. 2005). In *Drosophila melanogaster*, combinations of pair rule genes, Hox genes, and GATA factors work to specify the somatic gonadal precursors from the dorsolateral mesoderm of posterior abdominal segments, at around Stage 10 of embryogenesis (Moore et al. 1998; DeFalco et al. 2004).

Jury still out on Urbilaterian gonads

Beyond a mesodermal origin for the somatic structures of the reproductive system, no general pattern emerges from a phylogenetic consideration of these systems across the Bilateria. Convergent evolution of every aspect of the system is apparent not only among phyla, but also within phyla; this conclusion was also reached by Beklemishev (1964) in his comprehensive studies of comparative anatomy:

> ...we may assert that the great complexity of the genital apparatus, the appearance of the internal fertlisation, and the development of copulatory organs are characteristic of only the terminal branches of development and have arisen independently in each phylum of Bilateria and even in separate groups within each phylum. (Beklemishev 1964)

To date, the evidence for conservation of gene function in somatic gonad cells is tenuous, and limited to the protein product of a single gene. In fruit flies mutant for the *traffic jam* (*tj*) locus, the somatic gonad is specified correctly during embryogenesis, but ovarian morphogenesis in larval stages is disrupted (Li et al. 2003). Li and colleagues reported (as unpublished data) that the gonadal expression of mouse *tj*-like transcription factors suggests that this gene may have a similar function in both mouse and fly somatic gonads. Given the lack of support for molecular conservation thus far, I cannot suggest homology of molecular pathways involved in specification of the somatic gonad or in later gonadogenesis, consistent with repeated convergent evolution. In summary, while it is likely that

Urbilateria lacked a complex somatic reproductive system, it is at present impossible to speculate on whether or not it possessed a true gonad, let alone any other somatic adaptations for reproduction.

Conclusions

This brief review of what is known about the specification of bilaterian reproductive systems during development, suggests that we are still far from being able to speculate about the specific gonad structure and reproductive strategy of Urbilateria. I propose, however, that Urbilateria was unlikely to have had a complex somatic reproductive system, and that whatever somatic support it did have for gametogenic cells was of mesodermal origin. The changes in life histories undergone by lineages that have descended from the Urbilateria as they occupied different ecological niches, led to morphogenetic modification of these mesodermal derivatives, resulting in convergent evolution of different elements of somatic reproductive systems, including gonads, gonoducts and gonopores, copulatory organs, and adaptations for viviparity.

Acknowledgments

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