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Ontogenetic and phylogenetic aspects of the cephalopod circulatory system

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Summary. A brief description of the early embryonic development of the circulatory system in a representative of the coleoid cephalopods *(Octopus vulgaris)* is presented as an example of the rather uniform pattern observed at the outset of organogenesis. The role of the outer yolk sac both in morphogenesis and in the physiology of the embryo is emphasized. On the basis of recent observations reported in the literature, a preliminary comparison with the yolk sac of *Nautilus* is made, which in particular allows one to assess the relative importance of embryo size and bauplan constraints, respectively.

Key words. Cephalopoda; development; circulatory system; phylogenetic aspects.

Introduction

The embryonic development of cephalopods differs from that of all other molluscs; outstanding features are the bilaterally symmetrical (non spiral) superficial cleavage leading to a discoblastula, epibolic gastrulation, and integration of the large uncleaved yolk mass into a central 'yolk organ', which is distinct from the definitive digestive system. Early organogenesis is characterized by a virtually two-dimensional lay-out of organ areas that are gradually subdivided into distinct organ rudiments[10]. The juxtaposition of initially flat organ rudiments on the spherical surface of the yolk mass is transformed into a more compact organization during later organogenesis when the embryo cap gradually contracts and thus compresses the underlying yolk core. The yolk volume displaced by this contraction shifts into the outer yolk sac, which is a ventral 'outgrowth' of the headfoot. This extra-embryonic appendage is covered by a thin membrane of ciliated ectodermic cells underlain by a network of mesodermic elements that can be viewed as a greatly enlarged extension of the blastopore lip[10]. In large sized eggs, it is much greater in surface than the cap forming the embryo proper. In several groups of pelagic squids (e.g. Ommastrephidae), which produce very small eggs, the embryo is characterized by an extremely reduced, indeed rudimentary outer yolk sac. Except in these small sized embryos, the outer yolk sac plays a dominant role in the blood circulation of cephalopod embryos throughout organogenesis. Commonly, development of the circulatory system starts in this appendage of the headfoot.

Establishment of the yolk sac circulation

Soon after completion of the yolk sac envelope, a narrow hemal space appears between the envelope and the yolk syncytium[2]. Muscular elements differentiated from the scattered mesoderm-derived cells begin to contract periodically. These contractions are coordinated in such a way that a broad wave of contraction travels over the entire yolk sac surface, starting at the mouth side (fig. 1). The interval between individual waves of contraction is not strictly a function of traveling speed, which in turn may change with temperature[11].

The blood space surrounding the yolk syncytium under the envelope of the outer yolk sac rapidly extends, first into the mesodermic layer of the headfoot, then into the pallio-visceral zone (figs 1 and 2). The system of intra-embryonic sinuses is strictly symmetrical and shows smooth gradations from narrow spaces to tubular vessels, the first of which are the primitive paired vena cava tubes (fig. 3). Throughout early organogenesis, the blood is driven through these spaces by the peristaltic pulsations of the outer yolk sac envelope. Thus the nutrient

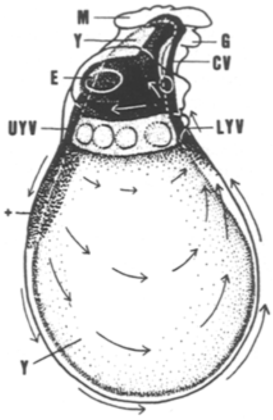

Figure 1. Organogenetic stage (stage X of Naef[10]) of *Loligo vulgaris* in lateral view, with semi-schematic presentation of hemal spaces in the embryo proper (white arrows indicate direction of blood flow). The arrows on the outer yolk sac symbolize the passage of a contraction wave, the area marked + being that of the strongest contraction of the yolk sac envelope (adapted from Portmann[11]).

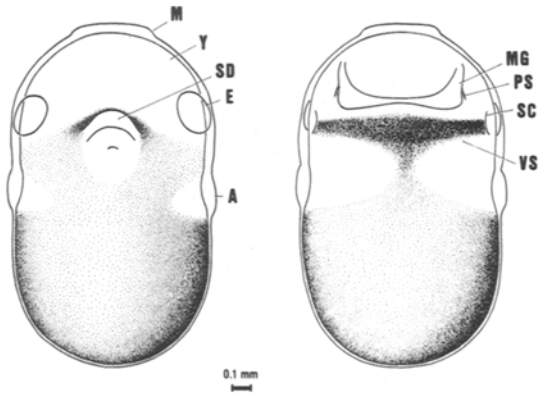

Figure 2. An early organogenetic stage (stage VIII of Naef) of *Octopus vulgaris*, in dorsal view at left, ventral view at right, with hemal spaces marked in dark (Figures 2–8 are reconstructions made from serial sections[2]).

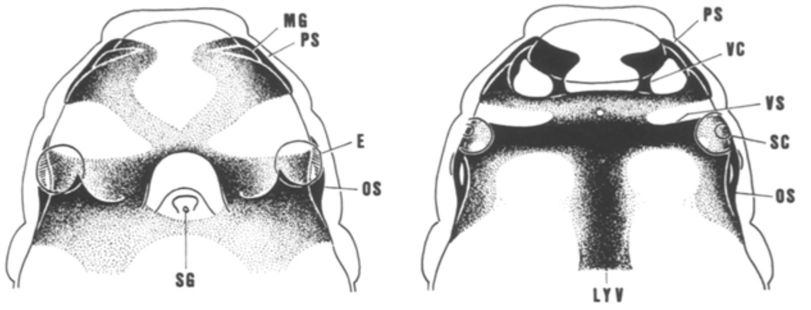

Figure 3. Stage IX of *Octopus vulgaris*, dorsal (left) and ventral view (right) of embryo proper.

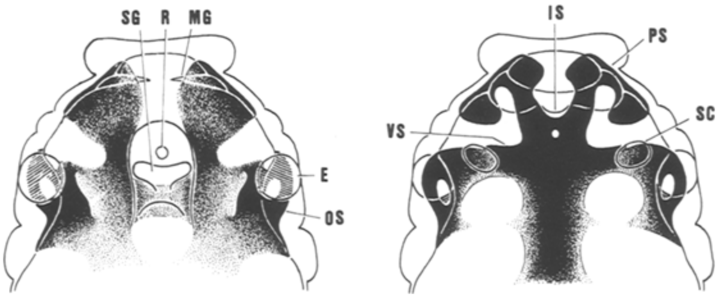

Figure 4. Stage X of *Octopus vulgaris*, dorsal (left) and ventral view (right).

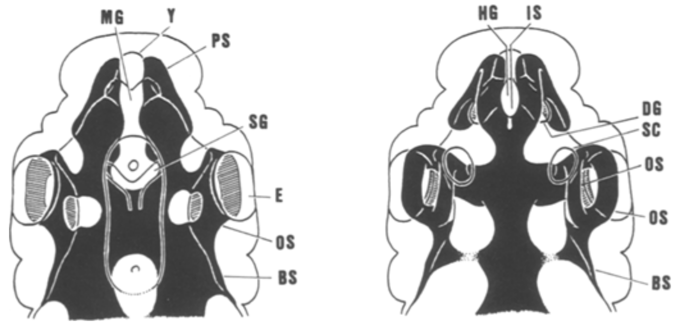

Figure 5. Stage XI of *Octopus vulgaris*, dorsal (left) and ventral view (right).

and oxygen enriched blood of the yolk sac sinus is pushed into the embryo proper through a broad ventral sinus, and from there it returns via the cephalic and the posterior sinuses to the dorsal side of the yolk sac (figs 4–7). Subsequent development reveals that the entire intra-embryonic system of sinuses and vessels established during early organogenesis becomes part of the definitive venous system of the animal. The initially lacunar ('schizocoelic') spaces differentiate into vessels and hemal spaces with an endothelial lining surrounded by a basal lamina and pericytes[4]. In contrast, the hemal space of the outer yolk sac remains lacunar, without any cellular barrier between the yolk syncytium and the blood. The situation found in the *Nautilus* embryo by Arnold and Carlson[1] is markedly different in that the outer yolk sac shows an elaborate system of blood vessels instead of a continuous hemal space.

Formation of the central circulatory organs

Early organogenesis leads to a largely definitive architecture of the body. The organs are still small and mostly in a very early state of histodifferentiation, yet their positional relationships will undergo only minor shifts during subsequent organ growth and differentiation. It is only towards the end of this early phase of organogenesis that the paired rudiments of the systemic heart become histologically distinct within the mesodermic mass that surrounds the innermost part of the yolk mass[7]. These rudiments are pulled together by the general contraction of the visceral complex, by which the clasp-like midgut rudiment (figs 5 and 6) closes in the median sagittal plane (thus reducing the posterior end of the yolk syncytium to a thin thread). In the systemic heart, a small lumen appears as soon as the fusion of the compact rudiments is

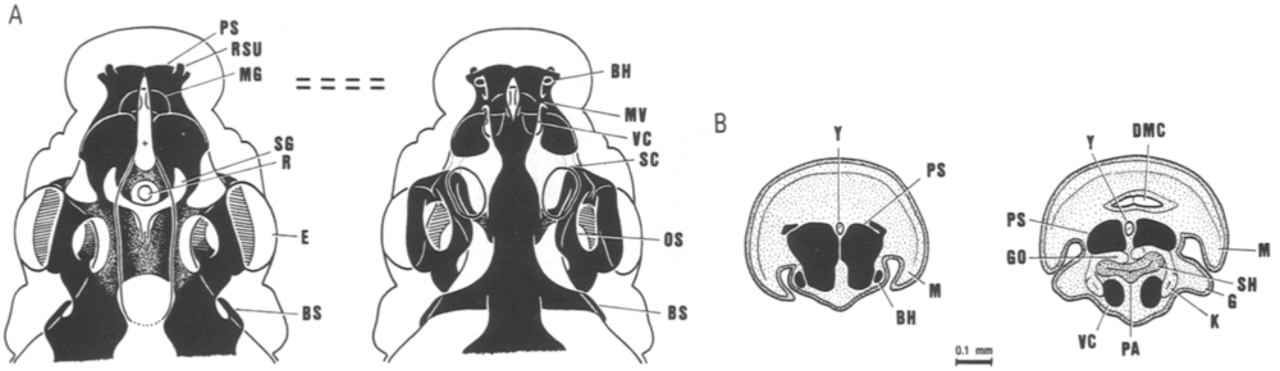

Figure 6. Stage XII of *Octopus vulgaris; A* dorsal (left) and ventral view (right); the two broken lines indicate the respective levels of the cross sections shown in *b*. upper line corresponds to the left figure of 6b, lower line to right figure of 6b.

complete. At about the same time, a lumen also appears on both sides within the mesodermic masses underlying the gill buds, marking the onset of branchial heart differentiation. Between these and the systemic heart lie the paired rudiments of the coelomic complex, which also begin to form lumina: kidney sacs and a pair of pericardial rudiments reach from either side around the systemic heart to the gonad (fig. 6b)[9]. The rudimentary branchial hearts begin to pulsate soon after they have formed a lumen, whereas the systemic heart becomes active only after its junction with the branchial hearts has opened on either side. Coordination and systolic/diastolic antagonism between the systemic heart and both branchial hearts is achieved only gradually once the connection between the central circulatory organs and the venous system is made.

Development of arterial/venous connections

While the outer yolk sac continues to pump the blood out of the dorsal head sinus and into the ventral cephalic vein, valves develop at the junction between the two posterior branches of that vein and the branchial hearts. The tiny gill rudiments are not yet vascularized, so the blood received from the cephalic vein is shunted directly to the systemic heart, from which two main arteries originate: the aorta cephalica and the aorta posterior (fig. 7), each of which very soon branches into secondary arteries (fig. 8). In coleoid cephalopods, the aorta cephalica is formed as an extension of the right end of the systemic heart, whereas in *Nautilus* it lies on the left side[8].

Three arterial/venous circuits are thus established: the mantle and visceral mass receive arteries from the posterior aorta ventrally, and from a dorsal vessel branching off the proximal part of the cephalic aorta (fig. 8). The main stem of the aorta cephalica provides the largest component of the early arterial system as it irrigates the entire headfoot, including individual arms. From the latter, the blood returns to the ventral sinus that receives the incoming blood from the outer yolk sac. At the time when this connection is achieved, the anterior part of the cephalic vein undergoes a thorough rearrangement related to the differentiation of the sub-esophageal part of the central nervous system.

The morphogenesis of new connections

The above-mentioned rearrangement is only one of several highlights of vessel 'plumbing' in cephalopod development. The apparent pacemaker of this process is the

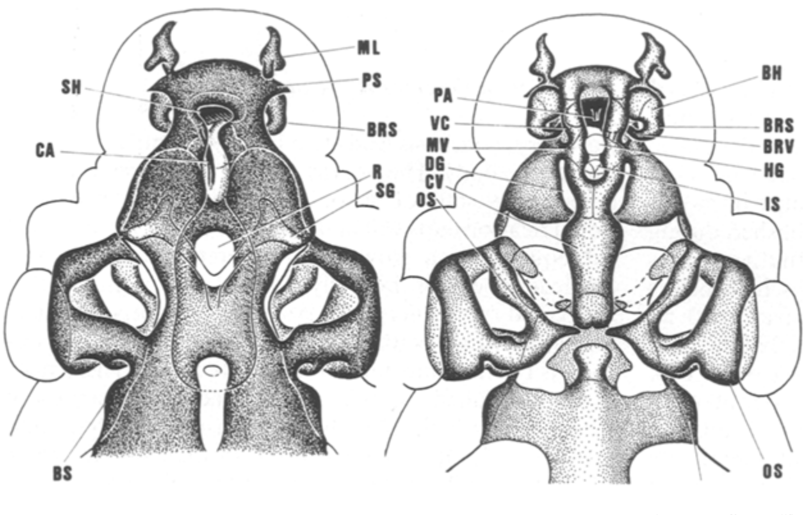

Figure 7. Stage XIII of *Octopus vulgaris*, dorsal (left) and ventral view (right).

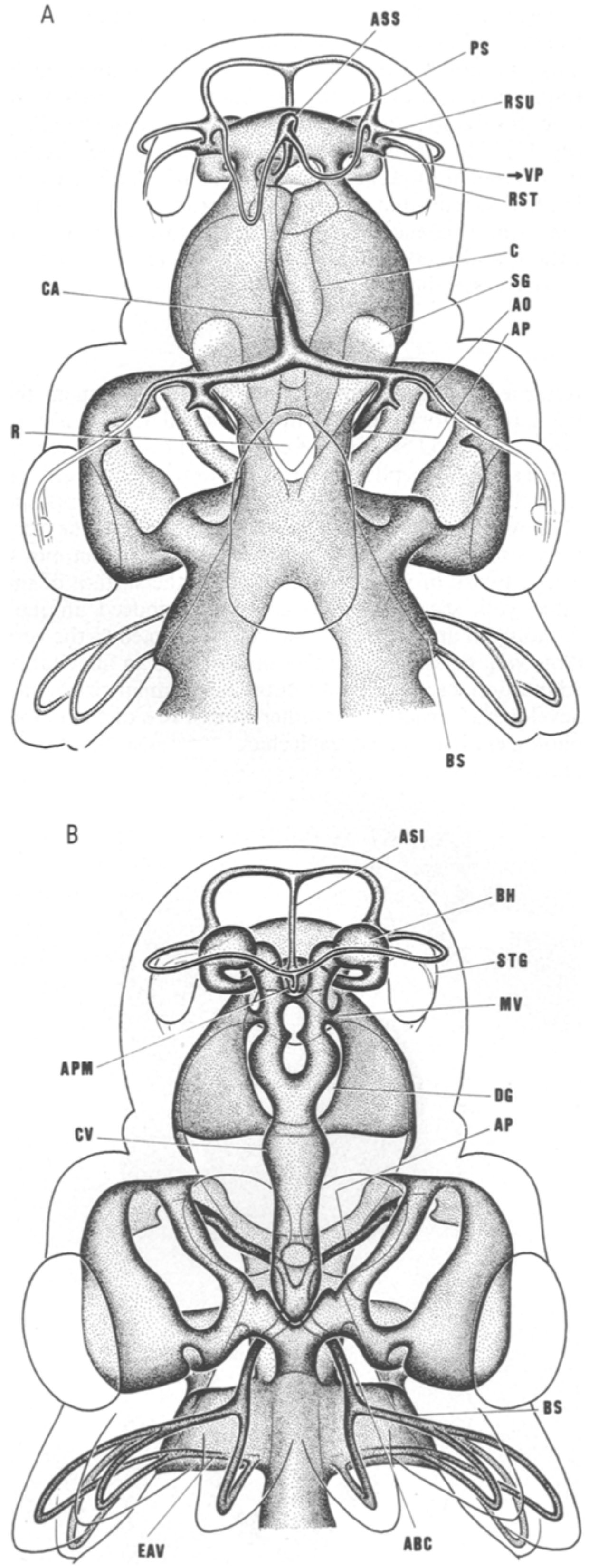

Figure 8. Stage XIV of *Octopus vulgaris; A* dorsal view, note closure of the mesenteric sinus (not marked) over the cepahlic aorta and the crop; *B* ventral view, showing the V-shaped dividing wall between the ophthalmic sinus of each side and the cephalic vein; note also the beginning fusion of the vena cava branches behind the intestine, at about the same level as the mesenteric veins enter them (cf. fig. 10).

concentration of the initially long subectodermic bands forming the primitive set of head ganglia. This concentration occurs in parallel with that of the midgut rudiment and later results in the drastic constriction of the yolk mass so that a small inner 'yolk sac' becomes very distinct from the outer sac. The ventral sinus receiving the blood from the outer yolk sac first increases in depth and then forms a new connection to the medial extensions of the ophthalmic sinuses. Into this Y-shaped bifurcation fits the lowermost extension of the actual cephalic vein (fig. 8b), it has also grown in depth due to the ganglion concentration. Finally the V-shaped dividing wall breaks open at its upper ends thus forming the definitive valve necessary to stop backflow once the cephalic vein starts pulsating.

A rather more complicated rearrangement occurs in the mantle complex where the pallial veins are formed from the lateral parts of the increasingly compressed posterior sinus (fig. 7, 8) and by differentiation of a secondary connection to each branch of the cephalic vein (fig. 9). The latter may fuse with each other behind the intestine. This connection remains open in *Nautilus* and in the Sepiolidae; in Octopoda it becomes the definitive vessel leading from the left branch (vena cava sinistra) to the right branchial heart while the primitive right branch disappears. In oegopsid squids, it is the right branch (vena cava dextra) that is conserved while the post-intestinal connection replaces the obliterated left branch (fig. 10).

Whereas these great connections are formed very regularly, subsequent branching patterns, especially in smaller vessels, tend to vary among individuals of a species. In more general terms, the initially symmetrical pattern of veins and sinuses becomes increasingly asymmetrical in certain areas of the body[6].

Advanced stages of yolk sac circulation

Except in some large sized octopus embryos (e.g. *Eledone moschata*)[4], the inner yolk sac remains compressed to small size only during a relatively short time. At the stages following organogenesis proper, it grows in size due to the transfer of yolk from the outer sac[11]. During this transfer, the circulation generated by the outer yolk sac envelope continues, and although the yolk neck is strongly compressed, the connecting sinuses still allow blood to pass. In decapods, the sinus surrounding the inner yolk sac differentiates a network of vessels that are integrated in the developing veinous system of the digestive tract[4]. In the incirrate octopods, the situation is somewhat different from that of the decapod embryos in that a large mesenteric sinus is formed from the dorsal and lateral parts of the inner yolk sac sinus (fig. 8).

Under undisturbed conditions, animals hatch out when the outer yolk sac is empty or nearly so (the envelope then is a small wrinkled appendage beneath the mouth). At that stage, the inner yolk sac may still be large; it will continue to supply nutrients via the circulatory system until the reserves are used up. This final resorption of yolk is independent of the incipient functioning of the digestive organs processing prey captured by the young animal[3].

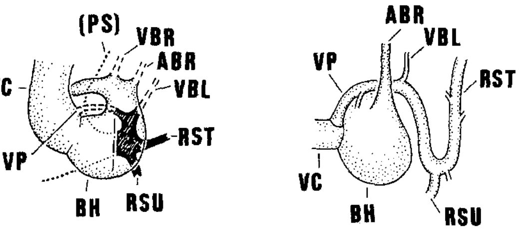

Figure 9. Schematic presentation of the derivation of the vena pallialis from the lateral parts of the posterior sinus in the embryo (left); the branchial vein is not indicated in the adult plan (right, after Grimpe[6]).

The influence of size on organ development

Ovum sizes in cephalopods vary from circa 0.8 mm to circa 25 mm according to species. The influence of egg size is recognizable from cleavage stages onward. In the smallest eggs, cleavage furrows may extend to the equator of the yolk mass so that the resulting discoblastula forms a cap covering most of the animal hemisphere. In contrast, the blastula stage in large eggs is represented by a flat disc of very limited extent. Subsequent expansion by epiboly of the cellular complex forming the embryo proper plus the envelope of the outer yolk sac thus is much stronger in these large sized germs. When comparing early embryos from different species characterized by very different egg sizes, one finds an inverse relationship between the absolute egg size and the size of the embryo proper relative to the entire yolk mass. However, when comparing absolute sizes of the embryo proper among different species, one nevertheless observes a clear difference. The embryo proper is indeed larger when the whole egg is larger compared to others, but this increase does not follow a simple proportionality.

Early morphogenetic stages are rather similar among species independently of the embryo size. In contrast, organ differentiation is influenced to some extent by the respective volume of organ rudiments, which are combinations of epithelial complexes and/or (more or less bulky) cell concentrations. One of the most drastic differences between small and large sized embryos exists in gill differentiation. In the smallest hatchlings (ommastrephid squids, and argonautids among the incirrate octopods), gills are still rudimentary and cannot yet function in respiration and excretion[12]. In contrast, in larger sized embryos the gills are fully developed long before hatching (fig. 11). The rheology of the developing blood circulation in cephalopods has not yet been analyzed; it is reasonable to assume that vessel differentiation must somehow represent a balance between increasing demand for irrigation, and blood pressure limitations due to blood viscosity/vessel diameter relationships.

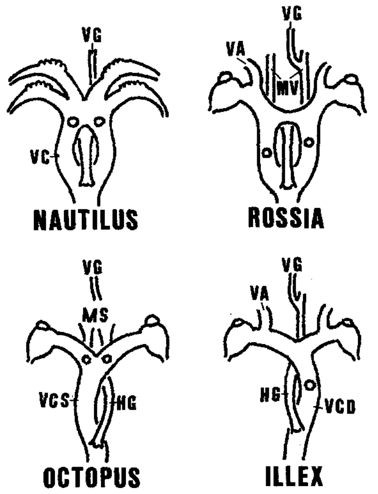

Figure 10. A schematic overview of the vena cava complex in *Nautilus* and in three coleoid cephalopods; the open circles represent the junction of the mesenteric vein to the corresponding vena cava branch (from Naef[8]).

Discussion

Retarded gill development (making the integument the main respiratory surface) in very small sized embryos provides a good example of convergent evolution resulting in similar morphological adaptations that are ultimately related to the limitation of gamete size. The opposite phenomenon, divergent evolution, appears in the very large sized embryos of *Nautilus* and of cirrate octopods. The network of vessels developing on the surface of the outer yolk sac in *Nautilus* embryos is indeed unique[1]. Although it appears to be a structure adapted to the very large yolk mass, it can not be explained as an 'inevitable' adaptation, because cirrate embryos of similar size do not develop this structure[5]. In other words, two distinct morphologies are observed, each characterizing a sub-class of cephalopods.

Figure 11. A newly hatched *Loligo vulgaris* (mantle length 2.8 mm), injected (from the cephalic sinuses) with a carbon suspension. Note the elaborate network of gill vessels.

Which one of these two morphologies is likely to represent the ancestral condition of the class Cephalopoda? In approaching this question, it is again egg-size that has to be considered. The earliest cephalopods recognized in the fossil record of the Upper Cambrian were small sized (1–2 cm) so that eggs similar in size to those of living *Nautilus* are simply inconceivable[13]. It is likely that these earliest cephalopods had eggs of a size similar to the smallest eggs known in living coleoids, i.e. somewhere close to 1 mm. In this case, the differentiation of a network of vessels in the outer yolk sac is highly improbable. The question to be considered next is whether the ancestral form of embryo had a distinct outer yolk sac at all. The living coleoids provide us with two alternative examples among the smallest known embryos, namely the ommastrephid squid *Illex* and the pelagic incirrate octopod *Argonauta*. At early organogenetic stages, the prospective outer yolk sac is an inconspicuous dome encircled by the arm rudiments and the buccal complex in both of these forms[10].

During the contraction of the embryo that characterizes later organogenesis, the yolk dome becomes smaller and smaller in the *Illex* embryo, whereas in *Argonauta* a distinct outer yolk sac is now formed by the partial extrusion of yolk from the embryo proper. As the *Argonauta* egg is even smaller than the egg of *Illex*, it is legitimate to conclude that the ubiquity of the distinct outer yolk sac at later organogenetic stages suggests this to be the common ancestral feature of the class. Wherever this character is conserved, the outer yolk sac envelope forms a pulsatile organ with an autonomous muculature. It becomes active, showing perfect coordination in a myogenic automatism comparable to heart functioning, long before the embryo proper has developed to the stage where definitive organs are functional. *Illex* embryos demonstrate that this system can be modified to the point of yolk sac elimination in later organogenesis (a modification that is inconceivable in larger sized eggs). However, the typical lay-out of organ rudiments necessitates the conservation of the early yolk dome.

In conclusion, the morphogenetic ground plan of cephalopods appears very uniform notwithstanding enormous differences in group or species specific egg size. Thus organogenesis starts under comparable conditions to become increasingly divergent only later on. The circulatory system reflects, both group typical morphologies and embryonic adaptations related to egg size, during its development.

Abbreviations used in figures: A, arm; ABR, arteria branchialis; AO, arteria ophthalmica; AP, arteria pedalis; APM, arteria pallialis media; ASI, arteria siphonalis inferior; ASS, arteria siphonalis superior; BH, branchial heart; BRS, branchial sinus; BRV, branchial vein; BS, brachial sinus; C, crop; CA, cephalic aorta; CV, cephalic vein; DG, digestive gland; DMC, dorsal mantle cavity; E, eye; EAV, embryonic arm vein ring; G, gill; GO, gonad; HG, hindgut; IS, ink sac; KS, kidney sac; LYV, lower yolk vessel; M, mantle; MG, midgut; ML, mantle lacuna; MS, mesenteric sinus; MV, mesenteric vein; OS, ophthalmic sinus; PA, posterior aorta; PS, posterior sinus; R, radula; RST, ramus stellaris of pallial vein; RSU, ramus superior of pallial vein; SC, statocyst; SD, stomodaeum; SG, salivary gland; SH, systemic heart; STG, stellate ganglion; UYV, upper yolk vessel; VA, vena abdominalis; VBL, vena branchio-linealis; VBR, vena branchialis; VC, vena cava; VCD, vena cava dextra; VCS, vena cava sinistra; VG, vena genitalis; VP, vena pallialis; VS, ventral sinus ('Gürtelsinus'[2]); Y, yolk.

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