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Dual origin of yolk nuclei in the lesser spotted dogfish, Scyliorhinus canicula (Chondrichthyes)

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ABSTRACT Using plastic embedding techniques and semithin sections in order to overcome the difficulties encountered in the sectioning of yolky eggs, we made a histological study of the developing blastodisc and early embryo of the oviparous dogfish Scyliorhinus canicula (L.) (Pisces: Chondrichthyes) from Roscoff (Brittany, France). According to our observations, several conclusions based on classical studies on elasmobranch development have to be revised as follows:

(i) Cleavage of the germinative disc into blastomeres lags considerably behind the mitotic activity of diploid nuclei derived from amphimixy. As a result, the number of surface blastomeres does not correspond in any way to the actual number of nuclei, even at the start of segmentation. Thus, segmentation is primarily syncytial.

(ii) The first set of yolk nuclei corresponded to the deepest nuclei that remained beneath the segmentation cavity after complete cellularization of the blastodisc. They became polyploid, some of them degenerated, and the others multiplied while remaining close to the extraembryonic endoderm during epiboly.

(iii) A second set of yolk nuclei was derived from the embryonic endoderm along its boundary with the extraembryonic endoderm. Endoderm cells escaped into the yolk layer nearby. They then give off free nuclei, which also became very large and finally were indistinguishable from the first set.

(iv) No yolk nuclei were derived from supernumerary sperm nuclei, even when the latter were able to divide several times in synchrony. Nuclei from this haploid lineage were always smaller than those in the first set and they were finally located in the deepest layer of the germinative disc, where they degenerated.

It is concluded that this chondrichthyan fish generates its yolk syncytium in a very different way from teleosts, in which all yolk nuclei are derived from the blastodisc.

Introduction

It has been claimed that the so-called "yolk syncytium" nuclei found around the yolk mass during elasmobranch development are derived, either in part or entirely, from supernumerary sperm, as a consequence of polyspermic fertilization (Rückert, 1890-1899). This claim has been included in some textbooks (Ziegler, '02; Pasteels, '58), while alternative interpretations have been put forward in others (Nelsen, '57; Starck, '65). We have now determined the origin and fate of yolk nuclei, showing that they are derived from two different sources, and not from supernumerary sperm nuclei. Our results resolve a classic controversy in elasmobranch embryology.

Materials and Methods

Several dozen eggs were used in the histological study. They were supplied by the Biological Station, Roscoff. Incubation was performed in Reims by the method of Mellinger et al. ('86). Some eggs were fixed immediately after dissection from captive females at the Biological Station. Developmental stages were defined as indicated in Table 1.

Fixation for histology was performed by immersion of open eggs in a mixture of 33% formaldehyde, 100% ethanol and glacial acetic acid (15:80:5, v/v), for 24 hours. Small portions were dissected out, dehydrated in 100% ethanol, cleared in propylene oxide and embedded in Epon 512 resin as described by Luft ('61). Series of 1.0 to 2.5-µm-thick sections were obtained with an OmU2 ultramicrotome (Reichert) equipped with glass knives. Sections were routinely stained with 1% methylene blue or toluidine blue in 1%

sodium borate. Other stains included McManus' periodic acid - Schiff reagent after removal of Epon by NaOH-saturated ethanol, and the presence of glycogen was controlled by digestion with amylase as described by Lillie (in Gabe, '68).

Using 2.5-µm-thick, serial sections, we determined the partial volume of each nucleus from a camera lucida drawing of its outline at a magnification of 1900x by cutting out and weighing the piece of paper in order to obtain the area in μm^2 by comparison to a reference area, and then multiplying by 2.5 µm to get the partial volume in μm^3. The total volume was obtained by adding up the partial volumes calculated from successive sections.

Results

Oviductal eggs

In order to determine the origin of yolk nuclei and to study their distribution and possible role in yolk utilization, we studied the development of eggs histologically from the very beginning, by using eggs enclosed in the nidamental glands that still had incomplete egg-cases. Their germinative disc was lens-shaped and 1.0-1.5 mm in diameter. It contained densely packed yolk granules which showed a more or less continuous gradient of diameters from the surface (3 µm), close to the thin vitelline membrane, to the bottom of the disc, where medium-sized granules (8 µm) appeared, changing to the elliptical, common yolk platelets that can be seen underneath (Fig. 1). No part of the germinative disc was free from yolk.

On live eggs, the germinative disc was bright orange in color and was surrounded by a zone of white yolk which probably corresponded to the medium-sized granules. The rest of the yolk varied from a yellow to a green color, depending on the female. The corresponding pigments were not seen in the semithin sections that were analyzed.

Fertilization was polyspermic, as described in other chondrichthyans. In mature females, live spermatozoa were only found in the nidamental glands, and the histological sections of germinative discs of eggs enclosed in these glands revealed several stages of fertilization. Thirty to fifty sperm nuclei were counted on individual serial sections. They were identified by their small

diameters (6-9 µm) and by their peripheral distribution, which was probably restricted to the white yolk, while the female pronucleus (10-12 µm) was located 150 µm beneath the vitelline membrane near the disc center, in the small-granule zone. Only two or three sperm nuclei remained near the female nucleus (Fig. 1A).

Table 2 shows the volumes (expressed in μm^3) of the various nuclei, as measured in serially prepared semithin sections. Nuclei of spermatic origin differed considerably in diameter from those of zygotic origin and from the female pronucleus itself. No confusion is possible at the relevant stages, denoted 1a through 2. Figure 2A shows one spermatic nucleus near the female pronucleus. This nucleus does not seem to have entered the S-phase since it is very small relative to the female pronucleus.

After amphimixy, which was not witnessed, the egg completed formation of its shell (i.e., its egg-case) and entered the caudal section of the oviduct. Thereafter, secretion of the anterior tendrils of the egg-case continued. When the length of the tendrils reached 10-15 cm, nuclei of zygotic origin and of sperm origin were still clearly distinguishable and located in two different areas, the first being central and the latter being peripheral. Zonation of the yolk in the disc was further emphasized at this time by the deposition of brownish yellow pigment and basophilic cytoplasm at the boundary between areas of small- and medium-sized granules. Nuclei from both sources divided synchronically, probably three times in all (Fig. 1B).

By the time the anterior tendrils were 40-50 cm long, most nuclei of the sperm lineage had disappeared. The remaining, presumably haploid nuclei were scattered in the areas of small- and medium-sized granules, but they never divided, while the zygotic, presumably diploid nuclei continued to divide in the central area (Fig. 1C).

Next, cell membranes were infolded from the surface of the central part of the disc, and this process allowed the first, open blastomeres to bulge outwards. Each blastomere contained one nucleus. However, all nuclei continued to divide actively, and the syncytial structure of the disc persisted. Some supernumerary sperm nuclei still survived at this stage.

Eggs equipped with complete tendrils remained for some time in the terminal part of the oviducts. Various stages of blastodisc

formation were evident. This process occurred by two different mechanisms: i) division of already closed, surface blastomeres, which reduced their sizes; and ii) production of additional, closed blastomeres at the surface of the unsegmented yolk. Production of additional lastomeres resulted from the orientation of several mitotic spindles towards the surface, and the infolding of plasma membranes around nuclei. Other nuclei continued to multiply by normal mitosis in the basal syncytium. Scattered supernumerary sperm nuclei were found in the deepest layer (Fig. 1D).

The increasing number of blastomeres led to the formation of a circular blastodisc, which was multilayered at its center and much thinner at its periphery. The blastodisc was eventually separated from the yolk by a shallow segmentation cavity (Figs. 1E, 3A).

Numerous nuclei remained in the superficial layer of the yolk, which was delimited by a continuous cell membrane: we refer to these nuclei as yolk nuclei. Their size and characteristics were still similar to those of blastodisc nuclei, but they had ceased to produce blastomeres (Fig. 3B). No supernumerary sperm nuclei survived at this stage, which corresponded to the egg-laying stage. Thereafter, the blastodisc flattened and blastomeres continued to divide actively. Yolk nuclei also continued dividing, soon forming clusters (Fig. 3C) and then merging in each cluster to produce a "giant nucleus" (Figs. 2C, 4, and Table 2). This process did not continue at later stages.

Epiboly

During stages A-D, the blastodisc forms two parts: i) the embryo, formed at the caudal margin, and (ii) the blastoderm, which corresponds to a still flat part of the blastodisc which begins to engulf the yolk through a process called epiboly, as also observed in teleosts. This process is completely independent of the "gastrulation" process. Thus, the blastoderm corresponds to the extraembryonic area, it is the anlage of the external yolk sac (EYS) and of the yolk stalk. Its endoderm is derived from the deepest cells of the blastodisc, while the embryonic endoderm is produced by a kind of "gastrulation" which proceeds under the embryonic shield and, therefore, cannot be seen from above. The EYS only closes during

stages I-K$_1$, and it is completely vascularized during stages L-M (Mellinger et al., '86).

Epiboly proceeded on a thin layer of cytoplasm that contained small yolk granules and lay above the deep yolk which contained the large yolk platelets. We call this superficial layer the yolk cytoplasmic layer (YCL), by analogy to that of teleosts, even though teleosts have no yolk particles in their YCL. Several types of giant yolk nucleus were observed close to the cell membrane of the YCL: i) the majority of these nuclei were very flat, their chromatin appeared dense and their extended outlines were often suggestive of amitosis; ii) others were multilobed, sometimes dividing by multipolar mitosis. Nuclear degeneracy also occurred at this level. It was recognized from broken or absent nuclear envelopes and scattered blocks of chromatin(Figs. 2C, 4).

During epiboly, organogenesis proceeds within the embryo, which begins to rise on the yolk (Figs. 2D, 5). The digestive cavity remains wide open in the direction of the yolk during stages G-H. It is derived from the archenteron, which is now closed. We observed a layer of yolk-free cytoplasm (YFC) upon the yolk that faced the digestive cavity. It constituted a kind of barrier between the two structures. Disruption of this layer, which occurs at stage P, probably initiates the entry of yolk into the gut.

At stages G-H, the boundary between embryonic and extraembryonic endoderm was located on the YFC (Fig. 5). Endoderm cells showed numerous examples of mitosis, as did cells in other epithelial sheaths (ectoderm, somatopleura, and splanchnopleura), which were also continuous from inside the embryo to the extraembryonic area. Cells of the extraembryonic endoderm were flat (Figs. 2D, 5), while those of the embryonic endoderm were cuboidal, and the latter part of the endoderm was even thicker along its lateral boundary. From this boundary, free nuclei and whole cells moved into the nearby part of the YFC. We can assume that whole cells provided additional free nuclei after some period of time. Many free nuclei had already become multilobed in shape and "giant" in size, and they were dividing in the YFC (Figs. 2D, 5, and Table 3).

Two populations of yolk nuclei are, thus ,present at the stages described above: i) the flat, primary yolk nuclei, derived from the blastodisc and located under the blastoderm, and ii) the secondary

yolk nuclei, derived from the endoderm. The production of secondary yolk nuclei stopped as soon as the yolk stalk had built up, at stages I-K. Once they ceased dividing, these nuclei were indistinguishable from the primary nuclei.

A typical and constant feature of yolk nuclei was their larger size, which most probably denoted an increase in DNA content. Volumes of yolk nuclei and their changes during development are shown in Tables 2 and 3.

Discussion

In elasmobranch fish, yolk nuclei are found opposed to or near the endoderm that covers the inner side of the EYS. Their origin and their role in digestion of the yolk have been unclear. Rückert (1890-1899) observed polyspermic fertilization in germinative discs ofTorpedo torpedo and Pristiurus melanostomus, fixed with sublimate-acetic acid, probably embedded in paraffin, and serially sectioned. He reported that there were generally 10-30 sperm nuclei in addition to the two pronuclei. After amphimixy, all these nuclei were assumed to divide synchronously several times, but this hypothesis was based largely on observations of groups of 4, 8 or 16 nuclei. Since such groups cannot be seen in single sections, we can assume that Rückert (1899) counted nuclei in serial sections, but this was not clearly stated in his report.

Sperm nuclei are generally located at the disc periphery, so they are recognized as a distinct population, at least in Pristiurus, which always has a number of them, while in Torpedo occasionally only one sperm nucleus is evident. In a reconstructed germinative disc of S. canicula at the 8-blastomeres stage, Rückert (1899, Fig. 32) depicted 55 such nuclei, which apparently formed open cells around the central area that contained the first blastomeres. Such sperm-derived blastomeres were considered to be incorporated into the extraembryonic sheaths. Rückert stated that these nuclei continued dividing beneath the disc, and that the nuclei of the yolk syncytium were all derived from them. He was unaware of the progressive degeneration that affects sperm-derived nuclei.

Rückert (1899, pp. 39-40) admitted that, in Pristiurus, he was really unable to identifiy sperm-derived nuclei (his "Merozyten")

among the normal segmentation nuclei (his "Furchungskernen"), since they had similar diameters ("Der Grössenunterschied zwischen den Merocyten- und Furchungskernen tritt bei Pristiurus nicht ganz so deutlich hervor wie bei Torpedo, und daher kommt es, dass bei ersterem Objekt die Unterscheidung der beiderlei Kerne, besonders wenn sie sich in verschiedenen Phasen der Mitose befinden, unmöglich werden kann"). However, he claimed that this identification was easy in Torpedo. Indeed, in his paper published in 1892, two camera-lucida drawings are presented to demonstrate a clear difference numbers of chromosome between "Merozytenkerne" und "Furchungskerne", with 2n being estimated to be about 36 chromosomes in Torpedo. This value is a considerable underestimate, since Stingo ('79) counted 86 chromosomes in T. marmorata and 66 chromosomes in T. ocellata. Moreover, Rückert (1899) admitted that the number of "Merozytenkerne" varied enormously in this genus (1-56 per egg), and he published several drawings in which such nuclei were completely absent.

There is another issue which is very confusing in Rückert's (1899) work: his interpretation of elasmobranch segmentation. Although he made several observations of syncytial segmentation, he always tried to ignore this phenomenon by referring to a so-called series of "normal" stages, i.e., stages of 2, 4, 8, ... and even 512 blastomeres in Torpedo ! Several of his own drawings clearly demonstrate that the reverse must occur: there was normally a considerable interval of time between cleavage (i.e., the formation of blastomeres) and prior nuclear division (see for example his Fig. 7, p. 46: Torpedo, showing the beginning of the first cleavage, with 8 nuclei already present).

Ziegler ('02) postulated that yolk nuclei could be derived from three different sources: supernumerary sperms, open blastomeres, and closed cells from the hypoblast. He supposed that every nuclei that entered the YCL would probably assume the same, abnormal aspect.

Beard (1878) observed polyspermy in Raja radiata, but he did not conceive of the possibility that yolk nuclei could be derived from a source other than the deepest, open blastomeres of the blastodisc, referring to the well known origin of these nuclei in teleosts. However, in teleost species that were later studied by Kopsch ('01-'11), only the trout blastodisc produced yolk nuclei from both deep

and peripheral, open blastomeres, while in the other species they were only produced by the peripheral blastomeres.

Doubts about Rückert's conclusions were recently expressed by Hamlett et al. ('87), and led us to reconsider the problem. Our conclusion is that Rückert's statement is completely false. He had been misled by the syncytial segmentation process found in elasmobranchs, which has been described by us in Scyliorhinus canicula. We admit that yolk nuclei come from two sources. The first source is the population of segmentation nuclei that remains beneath the blastodisc when the segmentation cavity appears. But there is also a second source, namely the endoderm of the embryo along its lateral boundary. This region was already depicted by Balfour (1878), but he stated that yolk nuclei formed cells that entered the endoderm and contributed to the build up of the ventral wall of the digestive cavity ! Our interpretation is indeed the reverse: cells, and even free nuclei, are detached from the embryonic endoderm along regions where it makes contact with the yolk. Later on, endoderm cells also migrate onto the YFC beneath the archenteric cavity in order to build up the ventral wall of the gut, but this migration is a distinct morphogenetic process. The detached endoderm cells enter the lateral parts of this cytoplasmic layer, and they are deeply embedded in it before they give off free nuclei. However, these new yolk nuclei never multiply in the YFC under the alimentary canal. Like the nuclei from the first set of yolk nuclei, they remain in close contact with the extraembryonic endoderm.

Our description of the early stages of development of this species of elasmobranch shows that several characteristics differ from those found in the development of teleosts: polyspermy vs. monospermy (confirming earlier studies); yolky vs. clear YCL and blastodisc; and lasting syncytial segmentation in the dogfish vs. direct production of the blastomeres in teleosts. It will be of interest to determine whether our conclusions can be generalized to all chondrichthyans and all teleosts.

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Literature cited

Balfour, F.M. (1878) A Monograph on the Development of Elasmobranch Fishes. Macmillan and Co., London.

Beard, J. (1878) The yolk sac, yolk and merocytes in Scyllium and Lepidosteus. Anat. Anz., 12:334-347.

Gabe, M. (1968) Techniques histologiques. Masson, Paris.

Hamlett, W.C., F.J. Schwartz and L.J.A. DiDio (1987) Subcellular organization of the yolk syncytial-endoderm complex in the preimplantation yolk sac of the shark, Rhizoprionodon terraenovae. Cell Tiss. Res., 247:275-285.

Kopsch, F. (1901) Die Entstehung des Dottersackentoblasts und die Furchung bei Belone acus. Internat. Monatsschr. Anat. Physiol., 18:43-127.

Kopsch, F. (1903) Art, Ort und Zeit der Entstehung des Dottersackentoblasts bei verschiedenen Knochenfischarten. Int. Monatsschr. Anat. Physiol., 20:101-124.

Kopsch, F. (1911) Die Entstehung des Dottersackentoblasts und die Furchung bei der Forelle (Salmo fario). Arch. mikr. Anat., 78:618-659.

Luft, J. (1961) Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol., 9:409-414.

Mellinger, J., F. Wrisez and M.J. Alluchon-Gérard (1986) Developmental biology of an oviparous shark, Scyliorhinus canicula. In: Indo-Pacific Fish Biology. T. Uyeno, R. Arai, T. Taniuchi and K. Matsuura, eds. Ichthyological Society of Japan, Tokyo, pp. 310-332.

Nelsen, O.E. (1957) Comparative Embryology of the Vertebrates. Blakinston Co., New York.

Pasteels, J. (1958) Développement embryonnaire. In: Traité de Zoologie. P.-P. Grassé, ed. Masson, Paris, vol. 13, pt. 2, pp. 1685-1754.

Rückert, J. (1890) Ueber die Entstehung der Parablast- oder Dotterkerne bei Elasmobranchiern. Sitzungsber. Ges. Morph. Physiol. München, 6:161.

Rückert, J. (1891a) Über die Befruchtung bei Elasmobranchiern. Verh. Anat. Ges., 5:253-254.

Rückert, J. (1891b) Zur Befruchtung des Selachiereies. Anat. Anz., 6:308-322.

Rückert, J. (1892) Über physiologische Polyspermie bei meroblastischen Wirbeltiereiern. Anat. Anz., 7:320-333.

Rückert, J. (1899) Die erste Entwickelung des Eies der Elasmobranchier. In: Festschrift zum 70. Geburtstag von Carl v. Kupffer, pp. 581-704, + plates 52-59 (As a separate issue: 124 pages + Tables I-VIII and legends)

Starck, D. (1965) Embryologie (2nd ed.). G. Thieme Verlag, Stuttgart.

Stingo, V. (1979) New developments in vertebrate cytotaxonomy. II. The chromosomes of the cartilaginous fishes. Genetica, 50:227-239.

Ziegler, H.E. (1902) Lehrbuch der vergleichenden Entwickelungsgeschichte der niederen Wirbeltiere. G. Fischer, Jena.

TABLES

TABLE 1. Developmental stages of S. canicula , and numbers of embryos studied, with some features that define each stage [1]

Stage	*Numbers of embryos*	*Sub-stages; main features*
1a	*6*	*Egg inside the nidamental gland; polyspermic fertilization*
1b	*7*	*Zygotic and some remaining spermatic nuclei dividing synchronously, free in the yolk*
1c	*7*	*First blastomeres. Zygotic nuclei still free in the yolk. No further spermatic mitoses .*
2	*6*	*More than 100 blastomeres, but still bulging*
3	*5*	*Numerous, flat blastomeres. Egg-case contained in the caudal part of the oviduct. No more spermatic nuclei. Continued formation of the blastomeres from the basal syncytial layer where free zygotic nuclei divide*
4	*16*	*Laid eggs. Stages preceding epiboly. Blastodisc separated from yolk by the segmentation cavity. Zygotic nuclei from the yolk no longer contribute to formation of the blastomeres, but they continue to divide*
5	*3*	*Beginning of epiboly. Yolk nuclei underneath.*
A	*5*	*Posterior thickening of the blastodisc. Yolk nuclei follow epiboly, the deeper nuclei showing endomitoses, some of them degenerating*
B-D	*5*	*From formation of the embryonic shield to enlargement of the head. Numerous mitoses in extraembryonic endoderm, as well as in the yolk nuclei, which often show abnormal mitoses*

E-H	*6*	*From formation of the medullary groove to first body motion. Migration of endoderm cells from the boundary between intra- and extraembryonic endoderm into the yolk cytoplasmic layer (YCL). Their nuclei become free, undergo endomitoses and merge into the population of giant yolk nuclei already present in the YCL*
I-Q	*13*	*Giant yolk nuclei in the YCL, either close to the extraembryonic endoderm or scattered among the most peripheral yolk platelets*

[1] *Sub-stages 1a through 1c refer to Figure 1 (A-C). For stages A through Q refer to Mellinger et al. ('86).*

TABLE 2. Volumes of yolk nuclei and of nuclei of some embryonic cell in S. canicula [1]

Stage	Spermatic nuclei	Female pronucleus	Zygotic nuclei in the yolk	Blastomere nuclei	Yolk nuclei	
					In the YCL	In the yolk
1a	320 ± 9.2 (70)	1,404 ± 62 (6)				
1c	1,189 ± 45 (44)		3,698 ± 322 (8)	4,558 ± 192 (12)		
2	208 ± 19 (20)		2,856 ± 91 (41)	3,076 ± 43 (33)		
3			2,138 ± 79 (24)	2,179 ± 71 (49)		
4 (early)			526 ± 13.4 (68)	673 ± 13.3 (35)	1,660 ± 92 (20)	
4 (late)				506 ± 4.2 (58)	939 ± 95 (15)	2,750-5,050 (23)
5				585 ± 3.6 (50)	2,160-6,725 (31)	
A				365 ± 3.2 (61)	2,074-6,480 (42)	

[1] *Data expressed in* μm^3 ± *SEM (number of nuclei measured), or by range. Volumes of yolk nuclei from stages 4 (late) through A were very scattered, so data ranges and numbers are indicated instead of the means. Stages refer to Table 1.*

TABLE 3. Comparison of volumes of nuclei in the endoderm and the yolk cytoplasmic layer (YCL) at stages E-H [1]

Endoderm nuclei		*Yolk nuclei in the YCL*	
Embryonic	*Extraembryonic*	*Beneath extra-embryonic endoderm*	*Beneath intestinal lumen*
515 ± 5.13 (75)	*395 ± 3.85 (119)*	*2,114 ± 35 (45)*	*3,620-11,770 (27)*

[1] *Data expressed as in Table 2.*

Legends

Abbreviations

b cells from the blastodisc or from the blastoderm
d presumably diploid nucleus, derived from the first zygotic nucleus
dn degenerating nucleus
ec ectoderm
en endoderm
l layer containing large yolk granules
m layer containing medium-sized yolk granules
me mesoderm
n1 peripheral yolk nucleus, with normal chromatin
n2 peripheral yolk nucleus, with half-condensed chromatin
n3 peripheral, possibly amitotic yolk nucleus, with condensed chromatin
n4 peripheral yolk nucleus, with condensed chromatin
n5 giant yolk nucleus freshly derived from the endoderm
n6 peripheral yolk nucleus similar to n1, but presumably derived from the endoderm
p layer containing yolk platelets
pl plurilobed nuclei
pz pigmented zone
s layer containing small yolk granules
sc segmentation cavity
tr tripolar mitosis
vm vitelline membrane
YCL yolk cytoplasmic layer
YFC yolk-free cytoplasm

Fig. 1. Five stages in the development of oviducal eggs, based on camera-lucida drawing of representative sections through the middle of germinative discs (A-C) or blastodiscs (D-E). In the drawings A-D, more nuclei than are actually visible in a single section have been shown. All nuclei, either at rest or dividing, are indicated by symbols: O and larger metaphase symbols indicate the female pronucleus (A)

and diploid nuclei (B-E), while dots (A,C,D) and smaller metaphases (B) indicate presumably haploid nuclei, derived from supernumerary sperms. Bars = 50 µm (bar A valid for A-D). A: Evidence for polyspermy. Two haploid nuclei are found near the female pronucleus, neither of them having already developed into a distinct male pronucleus. Several supernumerary sperm nuclei are scattered along the s-m transition zone. B: Synchronous mitoses, both involving diploid and haploid nuclei, separated by a transient zone of pigmented, dense cytoplasm. C: Asynchronous, syncytial segmentation. First plasma membranes appearing at the surface of the germinative disc. Small, haploid nuclei at rest remain deep in the s+m zone. D: Early blastodisc stage. Only one layer of surface blastomeres. Production of deep blastomeres by the syncytial parts of the germinative disc. Some haploid nuclei may still be found in the deepest parts of s+m+l zone (two of them are shown). E: End of blastodisc formation: egg-laying stage. Segmentation cavity first delineated above the basal syncytium, where diploid nuclei continue dividing but now only generate giant yolk nuclei. No more haploid nuclei.

Fig. 2. Micrographs of stained semithin sections. A: Female pronucleus (1) and sperm nucleus (2) at some distance amid yolk granules. The sperm nucleus has a nucleolus, a network of condensed chromatin, and a relatively small diameter. B: Stage 2. Distinct blastomeres at top. Mitosis of zygotic nucleus in the yolk. C: Columnar, epibolic blastoderm sliding over a layer of mesenchyme and over the yolk. A giant, flattened and extended nucleus shows several clumps of chromatin, suggestive of amitosis (arrowheads). D: Transition zone betweenthe embryo (in cross section, top left) and the extraembryonic area. Compare with Figure 4. The embryo is composed of cuboidal epithelial cells, while extraembryonic sheaths have flat cells (endoderm plus ectoderm are visible). Two giant nuclei can be seen in the yolk syncytium (arrows). Vitelline membrane still adheres to external surfaces. Bars: 20 µm.

Fig. 3. The first set of yolk nuclei. Camera-lucida drawings. Bar = 50 µm. A: Detail of Fig. 1E. Size gradient of yolk granules (black) in the s+m+l zone. Unlike yolk platelets, granules have irregular outlines in

semithin sections. B: Full set of scattered diploid nuclei resulting from final mitoses in the basal syncytium. C: Late stage, showing groups of 2-4 diploid nuclei, prior to merging.

Fig. 4. The first set of yolk nuclei (continued). Semi-diagrammatic sketches. Bars = 50 µm. A: The stage after that shown in Fig. 3C, showing giant, plurilobed yolk nuclei produced by the merging of diploid nuclei. Abnormal (tripolar) mitoses. The blastodisc has reached its maximal thickness (only its peripheral cells are shown). B: Epiboly (arrow). Thin blastoderm, showing a continuous surface layer and sparse deep cells. Peripheral yolk nuclei ($n_{1\text{-}4}$) show increasing condensation of the chromatin as they migrate over the yolk during epiboly, and they may divide by amitosis (see extended, n_3). Deeper nuclei are either plurilobed, dividing, or degenerating.

Fig. 5. The second set of yolk nuclei. Camera-lucida drawings of semithin sections cut transverse through embryos, at stages G-H, attached to their yolk substratum (black granules). Bars = 50 µm. A: General aspect. n_5, New, giant yolk nuclei resulting from merging of endoderm nuclei. n_6, New, giant peripheral yolk nucleus participating in epiboly. B: Detail of another section. Mitoses are not shown, but are common in all tissues and yolk.