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Evolutionary dissociation between cleavage, cell lineage and embryonic axes in sea urchin embryos

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Summary

Using vital dye staining and the microinjection of fluorescent cell lineage-autonomous tracers, the relationship between the first cleavage plane and the prospective larval dorsoventral axis was examined in several sea urchin species, including: *Strongylocentrotus purpuratus*, *S. droebachiensis*, *Lytechinus pictus*, *Clypeaster rosaceus*, *Heliocidaris tuberculata* and *H. erythrogramma*. The results indicate that there is no single relationship between the early cleavage pattern and the dorsoventral axis for all sea urchins; however, specific relationships exist for individual species. In *S. purpuratus* the first cleavage plane occurs at an angle 45 degrees clockwise with respect to the prospective dorsoventral axis in most cases, as viewed from the animal pole. On the other hand, in *S. droebachiensis*, *L. pictus* and *H. tuberculata*, the first cleavage plane generally corresponds with the plane of bilateral symmetry. There does not appear to be a predominant relationship between the first cleavage plane and the dorsoventral axis in *C. rosaceus*. In the direct-developing sea urchin *H. erythrogramma* the first cleavage plane bisects the dorsoventral axis through the frontal plane. Clearly, evolutionary differences have arisen in the relationship between cleavage pattern and developmental axes. Therefore, the mechanism of cell determination is not necessarily tied to any particular pattern of cell cleavage, but to an underlying framework of axial systems resident within sea urchin eggs and embryos.

Key words: cell determination, dorsoventral axis formation, cleavage pattern, evolution.

Introduction

Most sea urchin species display indirect development with the formation of a feeding, planktotrophic larva, called a pluteus. In these species early cleavage divisions and subsequent development leading to the formation of the larva proceeds in a stereotypic manner. Finally, after a prolonged period of growth, the larva settles and undergoes metamorphosis to the juvenile sea urchin.

Using various techniques it is possible to trace the development of individual blastomeres and establish a fate map relating their ultimate contributions in the resulting pluteus larva. Recently, such a fate map has been generated for the sea urchin, *Strongylocentrotus purpuratus* (Davidson, 1986; Cameron et al., 1987, 1989; Cameron and Davidson, 1991). During the course of their investigations, Cameron et al. (1989) observed that the prospective larval dorsoventral (aboral-oral) axis is oriented 45 degrees clockwise with respect to the first cleavage plane in the vast majority of *S. purpuratus* embryos, when viewed from the animal pole. Cameron et al. (1989) proposed that there is a causal relationship between the establishment of the first cleavage plane and the specification of the dorsoventral axis. They suggested that both may be set up relative to the path of sperm pronuclear migration within the zygote. In contrast with the results of Cameron et al. (1989) are those obtained earlier by Hörstadius and Wolsky (1936) and Kominami (1988) for the sea urchins *P. lividus*, and *Hemicentrotus pulcherrimus*. In these two species, no consistent relationship was seen between the first cleavage plane and the larval dorsoventral axis.

Wray and Raff (1989, 1990) showed that dramatic evolutionary changes have occurred in the relationship between cleavage pattern and the determination of specific cell fates. This is based on studies performed with Australian sea urchins belonging to the genus *Heliocidaris*. While one species, *H. tuberculata*, displays indirect development, the other, *H. erythrogramma*, displays direct development, without the formation of a feeding larval stage. In the latter case, metamorphosis to the juvenile sea urchin takes place in only four days post-fertilization. In *H. erythrogramma* the first three cleavage divisions proceed in the same manner as in indirect-developing species, but, the fourth cleavage division does not (Williams and Anderson, 1975; Raff, 1987; Wray and Raff, 1989). Instead,

the vegetal blastomeres divide in the same manner as the animal ones, and no micromeres are formed. The resulting 16-cell embryo contains animal and vegetal tiers of eight blastomeres, which are all of equal size. The fate mapping studies of Wray and Raff (1989, 1990) reveal that significant changes in cell lineage accompany these changes in cleavage pattern. In *H. erythrogramma*, the first cleavage plane always corresponds to the larval frontal plane, separating the embryo into prospective dorsal and ventral halves. Unlike indirect-developing sea urchins, most endodermal and mesodermal cells are derived from vegetal progeny of the prospective ventral blastomere.

Most evidence indicates that direct development in sea urchins is derived from an ancestral condition in which a feeding pluteus larva was formed (Strathmann, 1978). As the two *Heliocidaris* species diverged from a common ancestor within the last 10-12 million years (Smith et al., 1990; McMillan et al., 1991), dramatic changes have evolved in a relatively short period of time in the direct-developing species (Wray and Raff, 1989, 1990). These findings indicate that the early stages of development are not highly constrained, and changes in the relationship between cleavage pattern, developmental axes and cell lineage can occur.

Given these findings we sought to determine the extent to which the relationship between the first cleavage plane and the dorsoventral axis has changed during the evolution of sea urchins. We have used a combination of techniques to establish the relationship between the first cleavage plane and the prospective larval dorsoventral axis in a number of sea urchin species, including: *S. purpuratus*, *S. droebachiensis*, *Lytechinus pictus*, *Clypeaster rosaceus*, *H. tuberculata* and *H. erythrogramma*. We have found that dramatic differences exist, even between closely related species. Together, these results indicate that the underlying mechanism of cell determination is not tied to a particular pattern of cell cleavage divisions. Rather, cell determination appears to be tied to an underlying framework of developmental axes which are established early in the development of all sea urchins. This condition contributes to the tremendous evolutionary flexibility which is apparent in the early development of sea urchin embryos.

Materials and methods

Preparation of gametes

S. purpuratus and *L. pictus* were obtained from Marinus, Inc. (Long Beach, CA). *S. droebachiensis* were obtained from the Marine Biological Laboratory's Marine Resources Department (Woods Hole, MA.). *H. erythrogramma* and *H. tuberculata* were collected from coastal waters off Sydney, NSW Australia, and specimens of *C. rosaceus* were obtained from the Discovery Bay Marine Laboratory (Discovery Bay, Jamaica). Gametes were obtained following the procedures of Henry et al. (1989), Wray and Raff (1989) and Emlet (1986).

Vital dye labeling

In order to follow the relative position of the first cleavage plane through development, the vital dye Nile Blue sulfate was applied to a specific region on the surface of two-cell embryos as shown in Fig. 1a, following the techniques of Henry and Martindale (1987). In some *H. erythrogramma* embryos, this dye was also used to mark the relative position of the second cleavage plane in four-cell embryos (see Fig. 1b). Scoring is explained in the Results section.

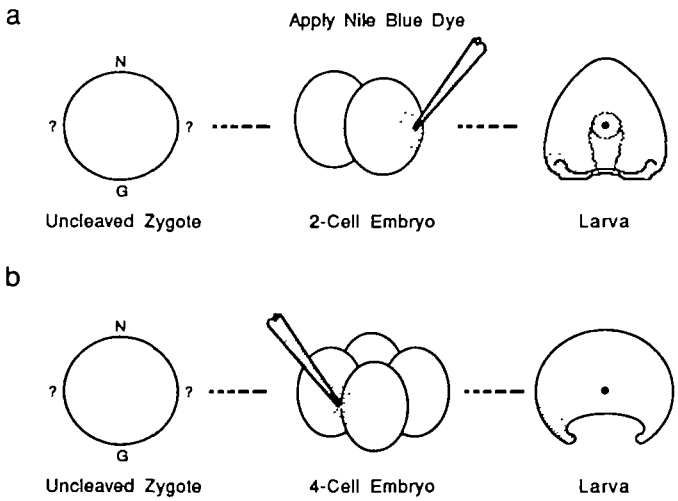


Fig. 1. Illustration depicting the placement of Nile Blue sulfate dye marks on two- and four-cell embryos to establish the relationship between the first cleavage plane and the prospective dorsoventral axis in the resulting larvae. Dye (shading) was applied either on the surface of one blastomere at the furthest point from the first cleavage plane in two-cell embryos (a) or in an equatorial location along the first cleavage plane in four-cell embryos (b). The locations of the animal (N) and vegetal (G) poles are shown on the zygotes. Representative larvae are drawn from an animal/dorsal perspective, where the ventral (oral) side is located towards the bottom and the opposite dorsal (aboral) side is located at the top. Thus the left side of these embryos is located to the right side of the figure and vice versa. The black dot indicates the center of the invaginated archenteron on the opposite, vegetal side of the larvae. In the resulting larva shown in (a), the dye mark is shown in one of the predominant locations for *S. purpuratus* embryos. In this case the dye is situated on the right/oral side, which includes part of the ciliated band and the far-right oral surface. In the resulting larva shown in (b), the dye mark is shown in one of the predominant locations for *H. erythrogramma* embryos. In this case the dye is situated on the right side and includes the right rim of the vestibule.

Microinjection of tracer dye

Microinjection of fluorescent dye was used to establish the relationship between the first cleavage plane and the larval dorsoventral axis in *L. pictus*. Eggs were washed several times and then fertilized in artificial sea water containing 8 mM p-aminobenzoic acid (PABA, Sigma, inc., St Louis) to inhibit hardening of the fertilization envelope. Prior to injection, two-cell embryos were electrostatically fixed to Petri dishes treated with 1% protamine sulfate. One blastomere in the two-cell embryos was then injected with approximately 2 pl of a solution containing lysyl tetramethylrhodamine-dextran (60-100 mg/ml in distilled water; D3306, Molecular Probes, Eugene, OR). In some cases, 33% glycerol was also included

in the dye solution. Following microinjection, the embryos were washed with fresh artificial sea water and cultured in the dark at 17°C. The locations of the fluorescent dye in the resulting larvae were used to determine the position of the first cleavage plane relative to the dorsoventral axis. The boundary between labeled and unlabeled regions corresponds to the first cleavage plane.

Analysis of development

The relative position of the first cleavage plane and the larval dorsoventral axis was determined in the young larvae (prism to early pluteus stages) in indirect-developing species, and in larvae with newly formed vestibules (after approx. 36 hours of development) in *H. erythrogramma*, when various larval symmetry properties could be clearly visualized. Echinoderm larval axes and symmetry properties are described in detail in the publications of Cameron et al. (1987, 1989) and Wray and Raff (1989, 1990). Here, the terms dorsal and ventral are used interchangeably with those of aboral and oral, respectively, for the larval stages of both indirect- and direct-developing species. It is important to note, however, that the larval dorsoventral axis is not coincident with that of the adult in indirect-developing species like *S. purpuratus*; though in direct-developing species like *H. erythrogramma*, the larval dorsoventral axis is coincident with that of the adult. Most observations were made on living specimens as it is difficult to preserve Nile Blue stain in fixed material. Microinjected larvae were fixed in a solution of 1% paraformaldehyde in sea water (pH 8.0) for 12 hours at 4°C. Following fixation, these larvae were washed twice in sea water and dehydrated through a graded series of ethanols for storage. Observations of fluorescence labeling patterns were then made in 90% glycerol in sea water.

Results

Relative location of the first cleavage plane in embryos stained with Nile Blue sulfate

Two-celled embryos of *S. purpuratus*, *S. droebachiensis*, *L. pictus*, *C. rosaceus*, *H. tuberculata* and *H. erythrogramma* were labeled with Nile Blue sulfate dye as shown in Fig. 1a. The number of cases examined, percent survival, and the locations of the dye spots within the resulting larvae are shown in Fig. 2a-f. Only those larvae with clearly visible staining were scored. The location of the dye in the resulting larvae, relative to the animal-vegetal and dorsoventral axes, was scored. One example is diagrammed in Fig. 1a for *S. purpuratus*. In this figure, as in Fig. 2a-g, the larvae are diagrammed from an animal/dorsal perspective, where the larval ventral (oral) surface faces towards the bottom of the figures. In this representative *S. purpuratus* larva (Fig. 2a), the dye is situated on the right/oral side, which includes part of the ciliated band and the immediately adjacent right/dorsal ectoderm. Twelve cases displayed this particular marking pattern out of a total of 45 cases examined, as recorded in Fig. 2a. Other labeling patterns were also seen and dotted lines point to respective positions where dye marks were observed in Fig. 2a. Predominant relationships between the first cleavage plane and the dorsoventral axis can be determined from the distribution of labeling patterns within the larvae. In *S. purpuratus*, most dye marks included cells of the right/oral ectoderm (12 cases), as mentioned above, or the opposite left/aboral ectoderm (11 cases). Thus, the first cleavage plane bisects the axis connecting these two symmetrical positions and is located 45 degrees counterclockwise with respect to the dorsoventral axis in most *S. purpuratus* embryos, as viewed from the animal pole.

In the case of *L. pictus* the vast majority of dye marks ended up in either the right/oral and left/oral ectoderm which included part of the ciliated band and adjacent dorsal ectoderm (14 and 13 cases, respectively, Fig. 2c). Unlike the cases seen with *S. purpuratus*, dye marks are generally absent in other regions of the larvae, notably in the aboral regions. Therefore, the prevalence of dye marks located on the left and right sides indicates that the first cleavage plane generally bisects the embryo along the plane of bilateral symmetry, as is also shown below in fluorescent dye injection experiments. Using this form of analysis, predominant relationships can be determined for the other species examined as well (see Discussion below).

The final dorsal and ventral location of dye marks applied to 2-cell embryos of *H. erythrogramma* (Fig. 2f) is entirely consistent with the fate mapping studies carried out by Wray and Raff (1989, 1990). In some additional cases lateral marks were applied on the first cleavage furrow in 4-cell embryos of *H. erythrogramma* (as shown in Fig. 1b). These marks were found to be positioned in the resulting larvae to the left and right sides, as expected (see Fig. 2g). These marks were located on either side of the opening of the vestibule, as is consistent with the fate maps generated by Wray and Raff (1989, 1990). These lateral marks were situated closer to the ventral side of the larvae since there is considerable stretching of the ectoderm towards the vestibule.

Intracellular injection of Lytechinus pictus *embryos*

The results of the dye marking studies presented above for *S. purpuratus* and *H. erythrogramma* were in close agreement with the results of previous cell lineage studies that employed the use of injected fluorochrome dyes (Cameron et al., 1987, 1989; Wray and Raff, 1989, 1990). In order to test further the efficacy of using Nile Blue sulfate dye, we injected one of the blastomeres of two-celled embryos of *L. pictus* for comparison. Typically 10 to 20% of the injected embryos survived and retained adequate fluorescence. A total of 55 injected embryos were analyzed. As the boundary between labeled and unlabeled regions corresponds to the first cleavage plane, the relationship to other axes can be determined in the resulting larvae. The results of these studies are shown in Figs 3 and 4a-h. The results were found to be in close agreement with those obtained from Nile Blue marking. In the majority of cases (60%) the first cleavage plane was found to pass through the plane of bilateral symmetry (Fig. 4a-d). This result is distinctly different from that found by Cameron et al. (1989) for *S. purpuratus* (see Fig. 5).

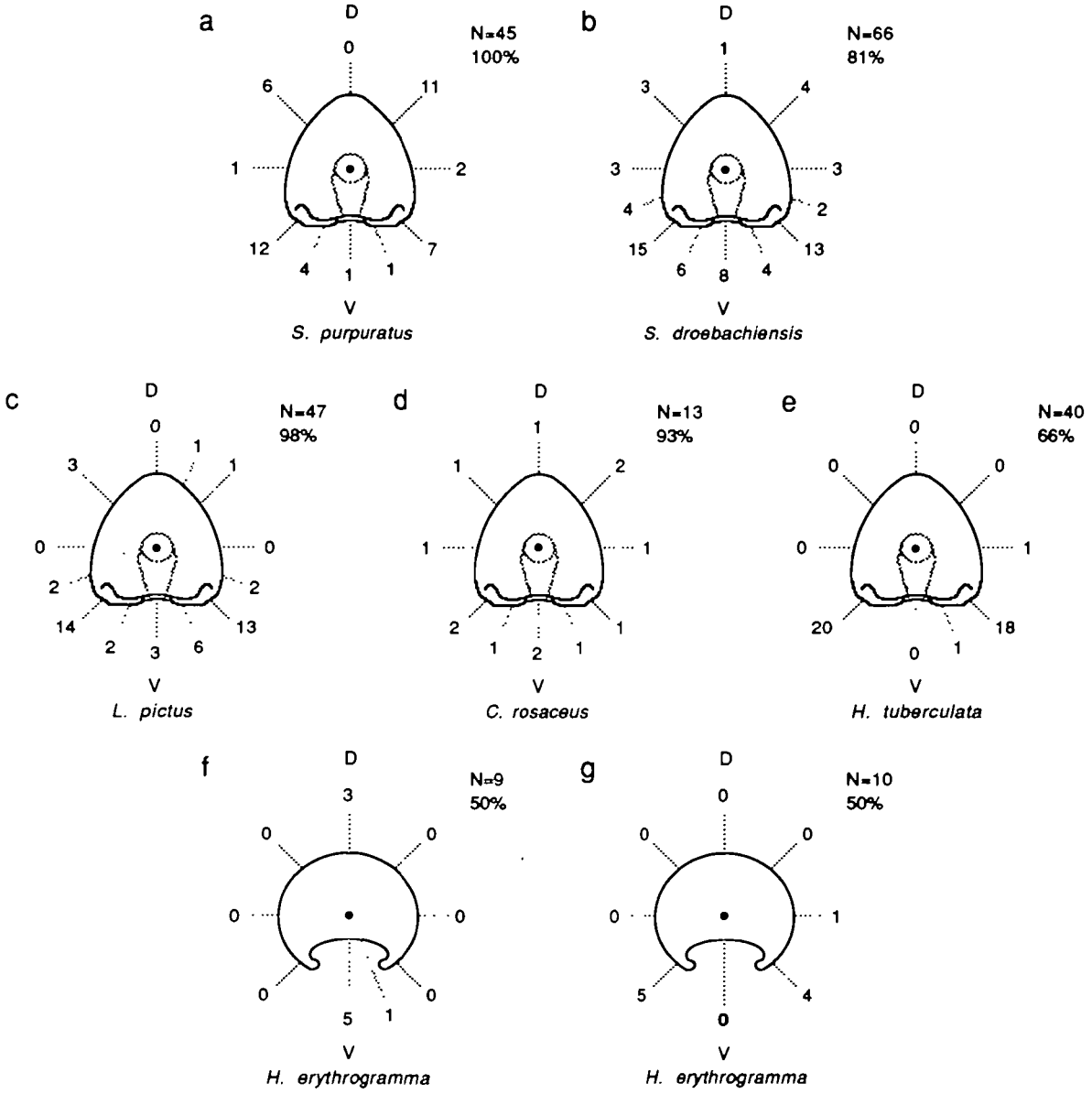
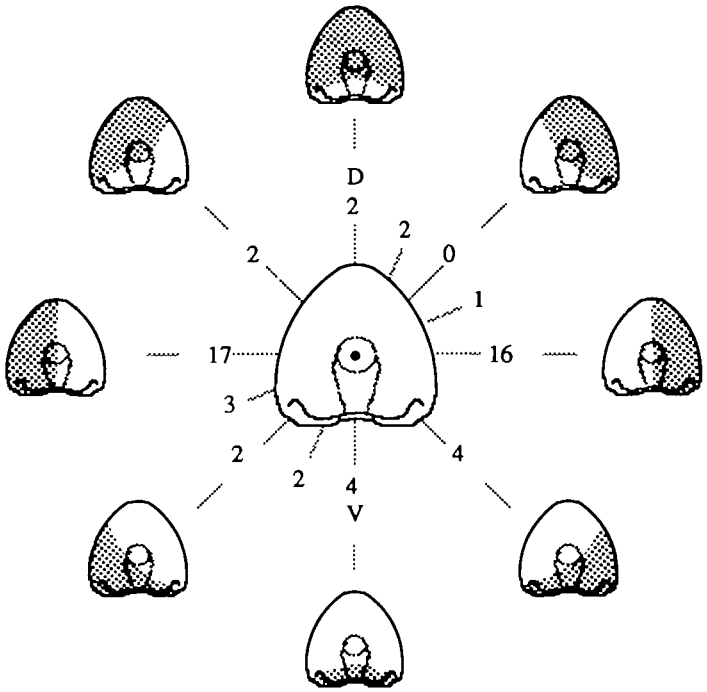


Fig. 2. Illustrations depicting the results of studies in which Nile Blue sulfate dye was used to mark (a-f) two- and (g) four-cell embryos of different sea urchin species. The embryos were marked as shown in Fig. 1. In (a-e) prism to early pluteus stage larvae are shown whereas the non-feeding larvae of *H. erythrogramma* are depicted in (f-g) containing the newly formed vestibular invagination. The resulting larvae are drawn from an animal/dorsal perspective, where the (V) ventral (oral) side is located towards the bottom and the opposite (D) dorsal (aboral) side is located at the top. Thus the left side of these embryos is located to the right side of the figure and vice versa. The black dot indicates the center of the invaginated archenteron located on the opposite, vegetal side of the larvae. For each case, the total number of embryos scored (N) as well as the percent survival is given. Individual numbers located around the periphery of each drawing record the number of cases displaying label in specific positions around the periphery of the embryos as drawn.

Discussion

The location of the first cleavage plane relative to the larval dorsoventral axis was determined from the data presented in Figs 2a-g and 3 as outlined in the Results section. Predominant relationships were observed for most species examined, and these are summarized in Fig. 6. Three other species have also been included in this figure based on the results of previous studies (*P. Lividus*, Hörstadius and Wolskey, 1936; *Hemicentrotus pulcherrimus*, Kominami, 1988; *Holopneustes purpurescens*, V. Morris, personal communication). In two of these latter species, *P. lividus* and *H. pulcherrimus*, the first cleavage plane was found to lie at any of eight different positions relative to the larval dorsoventral axis as shown in Fig. 5. A similar situation appears in embryos of *C. rosaceus*, although the number of cases we were able to examine was relatively small. In each of the other five species examined, fairly specific relationships were found to exist, but these were not all of the



L. pictus

Fig. 3. Illustration depicting the distribution of fluorescent dye in prism stage larvae of *L. pictus*. A total of 55 cases were scored. Specific fluorescent labeling patterns (shaded regions) are diagrammed and the number of cases observed are recorded for each type. The number of various intermediate labeling patterns are also shown. The orientation of the larvae is the same as depicted in Figs 1 and 2.

same pattern. The first cleavage plane passes through the frontal plane in the embryos of *H. erythrogramma* as well as those of *H. purpurescens* (V. Morris, personal communication). In the embryos of *S. purpuratus*, the dorsoventral axis is situated 45 degrees clockwise with respect to the first cleavage plane in the majority of cases and 45 degrees counterclockwise in most of the remaining ones, as viewed from the animal pole. A similar relationship was found by Cameron et al. (1989). In the remaining species examined the first cleavage plane was found to correspond with the plane of bilateral symmetry in the vast majority of cases. Together, the results indicate that no single relationship exists between the first cleavage plane and the larval dorsoventral axis for all sea urchin species. Furthermore, even amongst closely related species (i.e. *S. purpuratus* and *S. droebachiensis*, or *H. tuberculata* and *H. erythrogramma*), dramatic differences are found in the relationship between the early cleavage planes and the larval dorsoventral axis. Such evolutionary changes in the relationship between cleavage pattern and the underlying developmental axes has been proposed to account for the altered fate map of the *H. erythrogramma* embryo. (Henry and Raff, 1990, 1991).

Objections have been raised as to the use of Nile Blue sulfate dye as a marker in sea urchin embryos. Such lipidophilic dyes can diffuse through the plane of the cell membrane, but we did not encounter this problem in our studies. A claim has been made that vital dyes such as Nile Blue sulfate may have a dorsalizing effect if applied in great concentrations to sea urchin embryos (Lindahl, 1932a; Hörstadius, 1973). We did not observe such an effect in the course of this study. With moderate staining, Hörstadius (1973) claimed that there were no injurious effects from the dye. In the course of our experiments, great care was taken to keep the degree of staining to a minimum. Furthermore, the dye did not result in any increases in abnormal development or lethality. For the sea urchin species examined, predominant relationships between the first cleavage plane and the dorsoventral axis do not appear to be entrained as a result of the experimental manipulations. This is supported by the fact that there was not a prevalence of right/oral vs left/aboral staining in *S. purpuratus*, right/oral vs left/oral patterns in *S. droebachiensis*, *L. pictus* and *H. tuberculata*, or dorsal vs ventral labeling patterns in *H. erythrogramma*. The fact that the results of the Nile Blue staining experiments reported here are in agreement with those involving the microinjection of fluorescenated dyes (*S. purpuratus*, Cameron et al., 1989; *H. erythrogramma*, Wray and Raff, 1989; *L. pictus*, results reported here), supports the validity of this technique in establishing simple fate maps.

Depending on the species examined, there was a greater or lesser degree of variability in the distribution of the dye marks in the resulting larvae (Fig. 2a-g). In those species in which there was a highly consistent relationship between cleavage pattern and the dorsoventral axis, marks were clustered around the predominant positions. These cases are probably the result of slight differences in the exact placement of the dye in the two-cell embryos. On the other hand, more distant marks may represent either natural or artificially induced variation. It is known that physical perturbation can influence the position of the dorsoventral axis (Hörstadius, 1973). Although it is hard to separate these possibilities, it is likely that there is some natural variation in the relationship between the early cleavage planes and the dorsoventral axis within a single species. For instance, some species exhibit no predominant relationships (i.e. *P. lividus*, *H. pulcherrimus* and *C. rosaceus*). In addition, the results obtained from the microinjection of fluorescent dye in *L. pictus* also reveal some variation in the relative position of the first cleavage plane. Furthermore, some variation was noted by Cameron et al. (1989) for the sea urchin *S. purpuratus*. In that species, the dorsoventral axis was found to lie 45 degrees clockwise with respect to the first cleavage plane in 90% of the cases and 45 degrees counterclockwise in the remaining cases, when viewed from the animal pole. We observed the same predominant spatial patterns for *S. purpuratus* using Nile Blue sulfate dye, in which the dorsoventral axis was roughly 45 degrees clockwise with respect to the first cleavage plane in the majority of cases (approximately 60%), and 45 degrees counterclockwise in most other cases

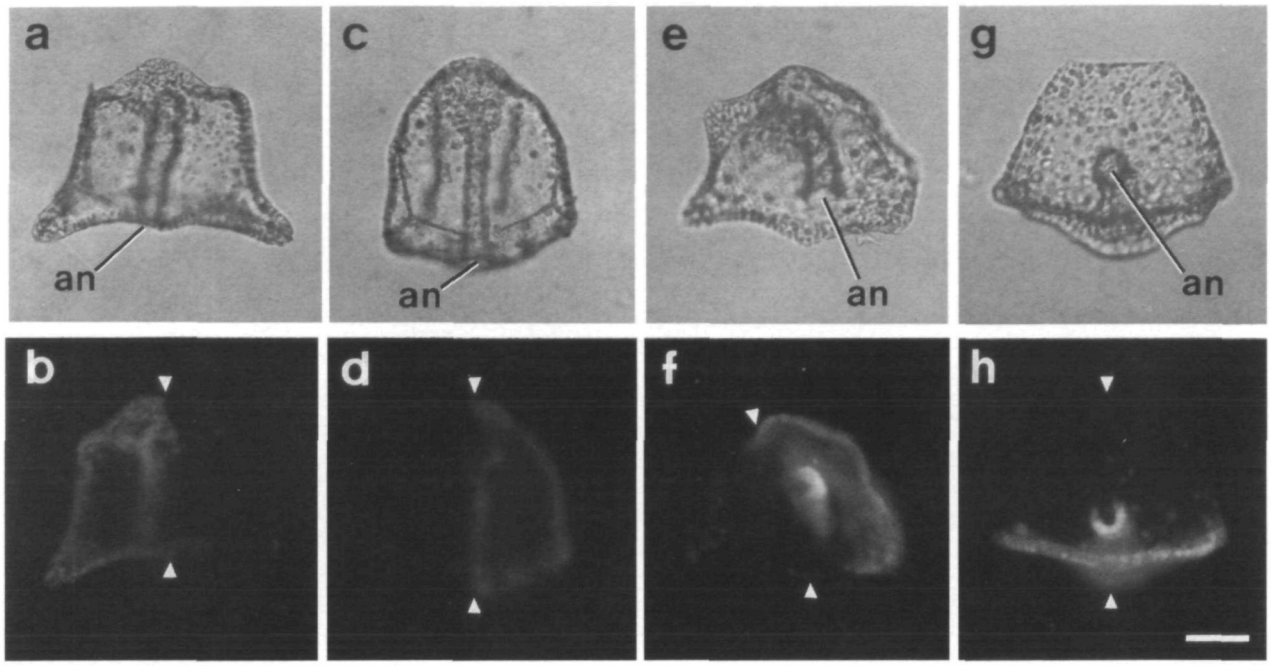


Fig. 4. Corresponding light and fluorescence micrographs showing various distributions of fluorescent label in prism stage larvae of *L. pictus* when a single blastomere was injected at the two-cell stage. The boundary between labeled and unlabeled regions represents the plane of the first cleavage division. (a,b) and (c,d) show the ventral (oral) surface of the larvae, where the vegetal pole is located at the bottom. The larva shown in (e,f) was also photographed from the ventral side, though it is turned slightly to the right, revealing a small portion of its left side. (g,h) show the vegetal surface, where the ventral surface is located at the bottom of the figure. In the majority of *L. pictus* embryos the first cleavage plane corresponded to the plane of bilateral symmetry and cells on either the right (a,b) or the left (c,d) sides of the larvae contained label. In some cases oblique labeling patterns were noted (e,f). In this case, cells located predominantly in the left aboral region of the embryo contained fluorescent label. In other cases, the first cleavage plane passed through the frontal plane, as shown in (g,h) where cells of the oral surface contained label. Arrowheads mark the plane of bilateral symmetry. an, anus. Bar, 50 μm.

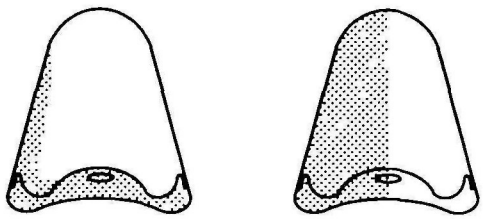


Fig. 5. Comparison showing differences in the typical relationship between the first cleavage plane and the larval dorsoventral axis in *S. purpuratus* and *L. pictus*, based on experiments involving the microinjection of fluorescent cell-lineage tracers. The pluteus larvae are drawn from an animal/dorsal perspective, where the ventral (oral) side is located towards the bottom and the opposite dorsal (aboral) side is located at the top. Boundary between the labeled (shaded) and unlabeled (unshaded) regions represents the first cleavage plane. Illustration of *S. purpuratus* is based on the findings of Cameron et al. (1989), while that of *L. pictus* is based on data presented here.

(approximately 31%), when viewed from the animal pole.

Dye marks applied to one blastomere at the point furthest from the first cleavage plane in two-cell embryos correspond to the lateral intersection of the second cleavage plane. As these marks are small, they do not label the entire progeny of a single blastomere from the two-cell embryo. Therefore, their ultimate location in the resulting larvae is subject to any distortions which may occur in the surface ectoderm during development. The more oral placement of the Nile Blue sulfate dye marks in the resulting larvae of *S. droebachiensis*, *L. pictus* and *H. tuberculata* indicates that stretching of the ectoderm takes place in these cases. There appears to be a disproportionately greater stretching of the dorsal (aboral) ectoderm relative to the ventral (oral) ectoderm. Such stretching was noted by Cameron et al. (1987) in *S. purpuratus*.

The results presented here, as well as the fate mapping studies of Cameron et al. (1987, 1989), indicate that there are fairly strict relationships between the first cleavage plane and the dorsoventral axis in several sea urchin species, including: *S. purpuratus*, *S. droebachiensis*, *L. pictus*, *H. erythrogramma*, *H. tuberculata* and also in *H. purpurescens* (V. Morris, in

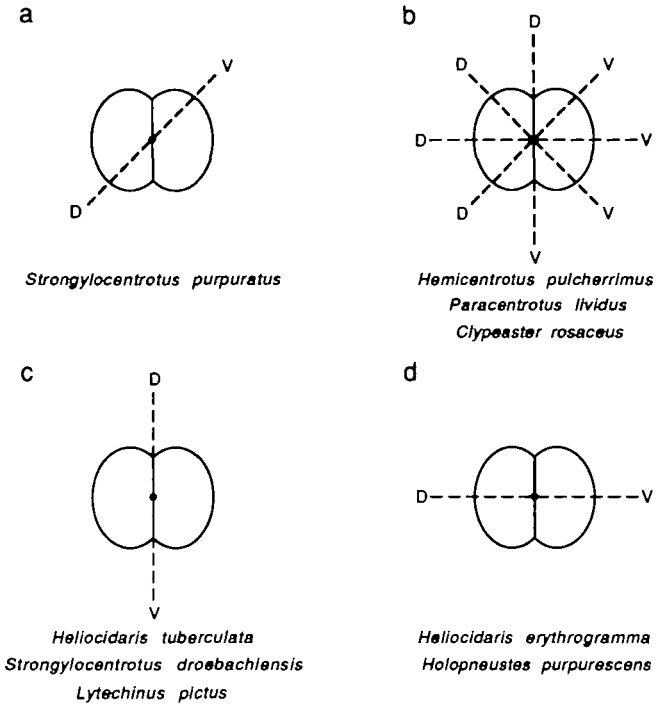


Fig. 6. Diagram of predominant spatial relationships between the first cleavage plane and the prospective larval dorsoventral (aboral-oral) axis among different sea urchin species. These relationships were determined from the data presented in Figs 2 and 3, and from previously published studies (*P. lividus*, Hörstadius and Wolskey, 1936; *H. pulcherrimus*, Kominami, 1989; *H. purpurescens*, V. Morris, personal communication). Two-celled embryos are diagrammed from the animal pole (black dot) and the prospective dorsoventral axis is shown as a dashed line. D, dorsal; V, ventral. The terms dorsal and ventral are used interchangeably with the terms aboral and oral, respectively, with regard to the larval stage of development. For indirect-developing species, the ventral pole corresponds to the site of the larval mouth. For direct-developing species (*H. erythrogramma* and *H. purpurescens*) the ventral pole corresponds to the site of the adult mouth, which forms within the echinus rudiment. No larval mouth forms in these direct-developing species.

preparation). On the other hand, the results of blastomere isolation experiments performed with indirect-developing sea urchins reveal that blastomeres isolated from two- and four-cell embryos are capable of complete regulation (Dreisch 1891, 1892, 1900, 1906; Boveri, 1907; Morgan, 1895; von Ubisch, 1925, Hörstadius and Wolsky, 1936; Henry and Raff, 1990). In addition, it has been shown that various physical perturbations can entrain dorsoventral polarity in young embryos (Runnström, 1925; Lindahl, 1932a,b; Hörstadius, 1938, 1973; Pease, 1939). Thus, the dorsoventral axis is not irreversibly fixed or determined at these early stages of development. The results of other investigations (Hörstadius, 1973; Henry and Raff, unpublished experiments) reveal that the dorsoventral axis is finally determined through subsequent cell-cell inductive interactions.

Although some sea urchin species exhibit a fairly constant relationship between the first cleavage plane and the dorsoventral axis, we argue that the mechanism of cell determination in sea urchins is not tied to any particular pattern of cell cleavage divisions (Henry and Raff, 1991). Rather, cell fates are assigned relative to an underlying framework of developmental axes which are laid down early in development. In some cases we observed that the first cleavage took place along what would usually have been the second cleavage plane, and vice versa. This was particularly true for *S. purpuratus*. This phenomenon was also recorded by Cameron et al. (1989). For most species examined, a variety of labeling patterns were, in fact, observed within single batches of embryos, including dorsoventral, oblique and bilateral patterns, as well as intermediate types (see Figs 2 and 3). These findings indicate further that the exact order or placement of cleavage planes relative to the dorsoventral axis is not critical for cell determination and normal morphogenesis.

The fact that there is a close connection between cleavage pattern and the various developmental axes in many species of sea urchins implies that some mechanism operates during normal development to conserve these relationships. Our findings indicate, however, that cell fate determination is freely dissociable from cleavage pattern, and this has occurred on many occasions during the course of evolution. We have also demonstrated that these processes are dissociable in an experimental context (Henry et al., 1990), since it is possible to change the early pattern of cell cleavage divisions without altering the position of the dorsoventral and animal-vegetal axes in *H. erythrogramma*. Such changes are possible in echinoids since cell determination proceeds, for the most part, epigenetically. The exact nature of these processes is not understood. The various developmental axes may serve to organize the subsequent expression of transcription-level controls of gene activity in the early embryos as proposed by Davidson (1989).

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