It is clear from the Tf genotypes that 1042C was the father of numbers 122801-122803, whereas 1135C fathered numbers 122804-122806. The presence of the a allele of Pgm in numbers 122801-122803 independently establishes 1042C as their father. Photographs of the Tf and Pgm electrophoretic patterns of parents and offspring appear in figure 1.

The male snakes involved in the experimental cross have quite different color patterns, reflecting their descent in part from different subspecies of *Lampropeltis getulus*. Thus it was of interest to see if the young could be segregated and assigned the proper paternity on the basis of their color patterns.

The parents and offspring are shown in figure 2, arranged according to relationships determined electrophoretically. Differences in the 2 classes of young were sufficient to enable the correct prediction of their male parents in a 'blind' test before the protein phenotypes of the young were determined. Without the latter data, however, the assessment of parentage would have been inconclusive. The best color clue to the identity of the male parent was the greater amount of light pigmentation in the young of 1042C, especially evident on the head. On the basis of its color pattern, the individual that died before hatching appears to have been fathered by 1135C.

There is no reason to think that the propensity of captive female kingsnakes for multiple matings differs from the situation among snakes in the wild. Indeed, multiple inseminations among these snakes seem more likely than in garter snakes *(Thamnophis)*, where a cloacal plug formed at copulation inhibits remating for a time at least[8]. Yet multiple inseminations occur abundantly in garter snakes, as seen from the proportions of melanistic and normal patterns in the broods of melanistic females inseminated in the wild[3]. The potential for detecting multiple inseminations in wild *Lampropeltis getulus* seemingly exists, for there are polymorphic populations whose genetics are at least partly understood[9,10]. However, there are few appropriate published data on the morph ratios in broods of known mothers, and clutch sizes are mostly too small for skewed morph ratios to be demonstrated convincingly.

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Morphogenetic effects of evans blue and of zinc ions in embryos of *Lytechinus variegatus*[1]

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Summary. Morphogenetic effects of evans blue and of Zn^{++} in *Lytechinus variegatus* embryos are described. Viable unhatched maximally-animalized embryos were induced with 4.2×10^{-5} M evans blue and with $4\text{–}8\times10^{-4}$ M $ZnCl_2$. Hatching inhibition was reversed only with $ZnCl_2$ suggesting that Zn^{++} is the preferred animalizing agent in this sea urchin species.

The application of certain chemicals to sea urchin embryos between early cleavage and the blastula stage produces either a hyperdevelopment of ectoderm (animalization) or exaggerated differentiation of endo-mesoderm (vegetalization)[2]. The polysulfonic acid dye, evans blue, and zinc ions (Zn^{++}) are two of the best chemical-inducers of animalization in whole sea urchin embryos[3-8]. When these animalizing agents are applied at weaker concentration, radialized (radially symmetrical) embryos are formed[2-6]. This report describes the morphogenetic effects of evans blue and of Zn^{++} in embryos of *Lytechinus variegatus* and the optimal conditions for induction of animalization and radialization in this sea urchin species.

Materials and methods. Methods for gamete collection, fertilization and culturing of *Lytechinus variegatus* embryos have been described[9]. The polysulfonic acid dye, evans blue, and Zn^{++}, as zinc chloride ($ZnCl_2$), each were applied to 2-cell *L. variegatus* embryos in both continuous- and temporary-treatment experiments. Experimental solutions of evans blue (EBS: evans blue solution) and of $ZnCl_2$ (ZnS: Zn^{++} solution) were prepared by dissolving the desired concentration of the respective chemical in SASW (artificial sea water containing appropriate concentrations of antibiotics)[9]. About 2×10^2 2-cell embryos were transferred to each Syracuse dish, containing a total 15 ml volume of solution. In continuous treatment experiments, 2-cell embryos were cultured continuously in experimental solutions. In temporary treatment experiments, 2-cell embryos were grown for 10–11 h in experimental solutions, washed 3 times in SASW and resuspended in 15 ml of SASW. Development proceeded at room temperature (23 °C). All experiments were performed at least in triplicate.

Results and discussion. Morphogenetic effects of evans blue in *L. variegatus* embryos are summarized in table 1. Animalization was induced by culturing 2-cell embryos continuously in 4.2×10^{-5} M, 2×10^{-5} M and 1.4×10^{-5} M EBS, while radialized larvae were found at 5.2×10^{-6} M EBS. At 4.2×10^{-5} M EBS, unhatched animalized embryos, ranging from type 3/4 to type 1/3 of Hörstadius[10] were observed, while at 2×10^{-5} M EBS, embryos showed a lesser degree of animalization. When control embryos were plutei, animalized larvae were unhatched, hyperciliated blastulae with laterally-extended and thickened ectodermal plates covered with long apical sterocilia, while radialized (radially symmetrical) embryos exhibited an ectodermal oral lobe,

Table 1. Morphogenetic effects of evans blue in *Lytechinus variegatus* embryos

EBS	Morphogenetic effect[a,b]							Effect on hatching[a]			Effect on hatching[b]		
	D	A (3/4)	A (1/2)	A (1/3)	INT	RAD	Ab-Pl	I	R	S1-R	I	R	S1-R
2×10^{-4} M	+	−	−	−	−	−	−	+	−	−	+	−	−
1×10^{-4} M	+	−	−	−	−	−	−	+	−	−	+	−	−
4.2×10^{-5} M	−	+	+	+	−	−	−	+	−	−	+	−	−
2×10^{-5} M	−	−	+	+	−	−	−	+	−	−	+	−	−
1.4×10^{-5} M	−	−	−	+	+	−	−	−	+	−	−	+	−
1×10^{-5} M	−	−	−	−	+	−	−	−	+	−	−	+	−
5.2×10^{-6} M	−	−	−	−	−	+	−	−	+	−	−	+	−
2×10^{-6} M	−	−	−	−	−	−	+	−	−	+	−	−	+
1×10^{-6} M	−	−	−	−	−	−	+	−	−	+	−	−	+

Two cell embryos were cultured in 'evans blue solution' (EBS) in both continuous- and temporary-treatment experiments. Abbreviations: (EBS), concentration of EBS; D, dead dissociated cells; A (3/4), A (1/2), A (1/3), type 3/4, 1/2, 1/3 in degree of animalization[10]; INT, intermediate forms between animalized and radialized larvae; RAD, radialized larvae; Ab-Pl, abnormal plutei; I, inhibition of hatching; R, retardation of hatching; S1-R, slight retardation of hatching. The plus sign (+) indicates the presence of, while the minus sign (−) indicates the absence of the effect on morphogenesis or hatching. [a]Results of continuous treatment experiment. [b]Results of temporary treatment experiment.

Table 2. Morphogenetic effects of $ZnCl_2$ in *Lytechinus variegatus* embryos

ZnS	Morphogenetic effect[a,b]						Effect on hatching[a]				Effect on hatching[b]			
	I-C	I-Mo	I-B	A (3/4)	INT	RAD	I	R	S-R	N	I	R	S-R	N
7×10^{-3} M	+	−	−	−	−	−	+	−	−	−	+	−	−	−
3.5×10^{-3} M	−	+	−	−	−	−	+	−	−	+	−	−	−	−
2.5×10^{-3} M	−	+	+	−	−	−	+	−	−	−	+	−	−	−
1.8×10^{-3} M	−	−	+	−	−	−	+	−	−	−	+	−	−	−
1.5×10^{-3} M	−	−	+	−	−	−	+	−	−	−	+	−	−	−
1×10^{-3} M	−	−	−	+	−	−	+	−	−	−	−	+	−	−
8×10^{-4} M	−	−	−	+	−	−	+	−	−	−	−	−	+	−
6×10^{-4} M	−	−	−	+	−	−	+	−	−	−	−	−	+	−
4×10^{-4} M	−	−	−	+	−	−	+	−	−	−	−	−	+	−
1.5×10^{-4} M	−	−	−	−	+	−	+	−	−	−	−	−	+	−
7×10^{-5} M	−	−	−	−	+	−	−	+	−	−	−	−	+	−
1.5×10^{-5} M	−	−	−	−	+	−	−	−	+	−	−	−	−	+
5×10^{-6} M	−	−	−	−	−	+	−	−	+	−	−	−	−	+

Two cell embryos were cultured in 'Zn^{++} solution as $ZnCl_2$' (ZnS) in both continuous- and temporary-treatment experiments. Abbreviations: (ZnS), concentration of ZnS; I-Cl, cleavage arrest; I-Mo, morula arrest; I-Bl, blastula arrest; A (3/4), 3/4 animalized larvae[10]; INT, intermediate forms between animalized and radialized larvae; RAD, radialized larvae; I, inhibition of hatching; R, retardation of hatching; Sl-R, slight retardation of hatching; N, normal hatching. The plus sign (+) indicates the presence of, while the minus sign (−) designates the absence of the morphogenetic or hatching effect. [a]Results of continuous treatment experiment. [b]Results of temporary treatment experiment.

lacked a mouth and contained a tripartite archenteron, surrounded at its base by mesodermal skeletal spicules and pigment cells. Intermediate concentrations of EBS produced intermediate forms between typical animalized and radialized embryos. Concentrations of EBS greater than 4.2×10^{-5} M resulted in eventual lysis and death, while concentrations of 2×10^{-6} M and 1×10^{-6} M produced abnormal plutei. The concentration of EBS which induced maximal animalization in *L. variegatus* embryos also produced the undesirable side effect of severe inhibition of hatching, which could not be reversed by return to SASW (temporary treatment experiment).

Table 2 summarizes the results obtained when *L. variegatus* embryos were cultured in experimental solutions of ZnS. Continuous treatment of 2-cell embryos with 4×10^{-4} M, 6×10^{-4} M and 8×10^{-4} M ZnS produced viable unhatched maximally-animalized embryos (type 3/4)[10]. When these animalized ambryos were returned to SASW after 10–11 h (temporary treatment experiment), the inhibition of hatching was reversed and the embryos hatched. The effect of higher concentrations of ZnS (7×10^{-3} M to 1×10^{-3} M) ranged from cleavage inhibition (at 7×10^{-3} M ZnS) to animalization with reduced viability and early embryo degeneration (1×10^{-3} M ZnS). Typical radialized larvae were observed at 5×10^{-6} M ZnS. Intermediate concentrations of ZnS induced morphogenetic effects intermediate between animalization and radialization.

The morphogenetic effects of evans blue and of Zn^{++} were similar in both continuous- and temporary-treatment experiments (tables 1 and 2). In contrast, while hatching inhibition produced in evans blue-induced animalized embryos was irreversible (table 1, column 4), that produced in Zn^{++}-induced animalized embryos was reversible (table 2, column 4). Viable hatched maximally-animalized[10] embryos of *L. variegatus* were produced by culturing 2-cell embryos for 10–11 h in $4–8\times10^{-4}$ M $ZnCl_2$, followed by transfer to SASW. These data suggest that Zn^{++} is the preferred animalizing agent in this sea urchin species.

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