

The embryos with supernumerary segments are induced by the treatment with hydroxyurea in the horseshoe crab

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Mankind has been interested in homologous structures in a individual such as body segments, appendages and fingers. But, the mechanism of determination of their number and differentiation has not been clear.

The authors found that hydroxyurea (the inhibitor of DNA synthesis) changes the differentiation of body segments of the horseshoe crab and increases the number of segments. We report the effect of hydroxyurea to segmentation of the horseshoe crab.

The embryos with supernumerary segments were induced under the following conditions:

[STAGE] From stage 12 (the stage of appearance of cephalothoracic segments) to stage 18 (the stage after the first embryonic moulting).

[TREATMENT TIME] more than 12 hr.

[CONCENTRATION OF HYDROXYUREA] 10^{-3} to 5×10^{-2} M.

Under above-mentioned conditions, the monsters were obtained at the rate of 80 to 100%. The rate of dead was about 0%. In normal sea water, no embryo with supernumerary segments was found in the examined 13,904 embryos.

The change of differentiation and the increase of number were shown in the abdominal segments. The total number of segments was +0 (106 – 6.5%), +1 (1,472 – 89.6%) and +2 (64 – 3.9%).

We show segments of normal embryos as the following symbols. The last (6th) cephalothoracic segment is shown as “T”. The first abdominal segment has very small appendages chilarias. It is shown as “C”. The second abdominal segment has large abdominal appendages. It is shown as “A”. The 3rd abdominal segment has brachial appendages. It is shown as “B”. The 4th abdominal segment has under-developed brachial appendages. It is shown as “b”. The horseshoe crab embryos have also three segments with the primordia of appendages. They are shown as “p”. The type of normal embryos and the embryos with supernumerary segments induced after the treatment at each stage were as follow:

[NORMAL EMBRYOS]	T – C – A – B – b – p
(stage 12 to 13)	T – T to C – C to A – A to B – B to b – b – p
[stage 14]	T – C – C to A – A to B – B to b – b – p
[stage 15]	T – C – A – A to B – B to b – b – p
[stage 16]	T – C – A – B – B to b – b – p
[stage 17]	T – C – A – B – b – b – p

“A to B” means the structure between A and B. By the treatment at stage 18, we obtained the embryos with supernumerary primordia. They can be shown as T – C – A – B – b – p – p.

From the examination of characteristics of the embryos with supernumerary segments, we suggest following things.

(1) The formation of primordia of cephalothoracic segments is finished till stage 12. The number of cephalothoracic segments is determined at this stage.

(2) The primordium of the 1st abdominal segment (C) is formed at stage 12 to 13. The 2nd one (A) is at stage 14. The 3rd one (B) is at stage 15. The 4th one (b) is at stage 16. The 5th one (p1) is at stage 17. The 6th one (p2) is at stage 18.

(3) When embryos are treated by hydroxyurea, the forming primordium differentiates into the structure between the anterior segment and itself.

(4) The differentiation of segments depends on the state of differentiation of anterior segments. In other words, the state of each segment successively determines the differentiation of next segment.

(5) The whole number of segments is determined as the results.