measured in the crude extracts in homogenization buffer, final volume 50 µl, containing 20 µM 8-$^3$H-cAMP or 20 µM 8-$^3$H-cGMP respectively. The samples were incubated at 37 °C for 15 min and the reaction was stopped by the addition of 30 µl of 10% TCA. cAMP and 5′-AMP or cGMP and 5′-GMP were separated on ‘Silufol 254’ TLC plates, using a solvent containing isopropylalcohol, 25% water solution of NH$_4$OH and water (7:1:2)[6]. The nucleotides were detected under UV-light, the spots were scraped off and radioactivity (dpm) counted in toluol scintillation fluid using a liquid scintillation counter (‘Rackbeta’, LKB). Protein was determined by the method of Lowry et al.[7].

*Results.* The data obtained demonstrate a remarkable, more than 2-fold increase on the cAMP PDE activity in the development of the embryo from stage 1 to stage 2 (fig. A). During the gastrulation (stages 3 and 4 embryos) the cAMP PDE activity in the epiblast remained practically constant at the level reached in the stage 2 embryo, whereas the enzyme activity decreased in the primitive streak and in the meso- and endoblast. In the latter cells, the level of the cAMP PDE activity was about 4 times lower than in the epiblast. In fact it means that a gradient of cAMP PDE activity (epiblast → primitive streak → meso- and endoblast) develops in the embryo during gastrulation.

As shown in figure B the cGMP PDE activity increased steadily during the development of the chick embryos from stage 1 to stage 4. The differences in the cGMP PDE activity between the different germ layers were smaller in comparison to those of the cAMP PDE. Unlike the cAMP PDE activity, no clearcut gradient of cGMP PDE activity was formed in the embryo during gastrulation.

*Discussion.* Taking into account that cells move stepwise during the primitive streak elongation[8], and also the suggestion that cAMP acts as an attractor in primitive streak elongation[2,4], it could be assumed that the mechanism underlying the process of gastrulation of the chick embryo is similar to that functioning in the slime mold *Dictyostelium discoideum* development[9]. In slime mold development starvation leads to the formation of the center of aggregation which emits waves of cAMP, and the aggregation of the cells occurs in the field of the propagating waves, the cells moving by jumps. In the case of the chick embryo the ‘center of aggregation’ could be located in (or near) Hensen's node which attracts the cells of the epiblast, thus forcing them to move ’downward‘ through the Hensen node.

In the case of *Dictyostelium,* high activity of cAMP PDE is necesssary for the decrease of the concentration of cAMP during the propagation of the waves of the attractor. Higher activity of cAMP PDE in the epiblast in comparison to that in mesoblast and endoblast is in agreement with the assumption made above.

The decrease of cAMP PDE (and also cGMP PDE) activity in the primitive streak could be related to the increase of catabolic activities in this area. Karner and Leikola[10] showed that the activity of the lysosome enzyme acid phosphatase increases in the primitive streak. Also it has been shown in our laboratory that the RNA and protein synthetic processes which could compensate the loss of the cellular material are less intensive in the meso- and endoblast in comparison to those in the epiblast (unpublished results).

*Conclusion.* Formation of a gradient of cAMP PDE activity in chick embryo during gastrulation was observed. The gradient observed could be considered as an additional evidence supporting the hypothesis according to which cAMP participates in the morphogenesis of the chick embryo.

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## Inheritance, maternal influence and biochemical analysis of an egg color polymorphism in *Ophryotrocha diadema**

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*Summary.* In the Polychaete worm *Ophryotrocha diadema,* the yellow coloration of egg yolk is due to the selective uptake of lutein from food. The genetic control of this mechanism depends on a single locus with 2 alleles, the dominant *Y* (yellow) allele, and the recessive *y* (absence of coloration) allele.

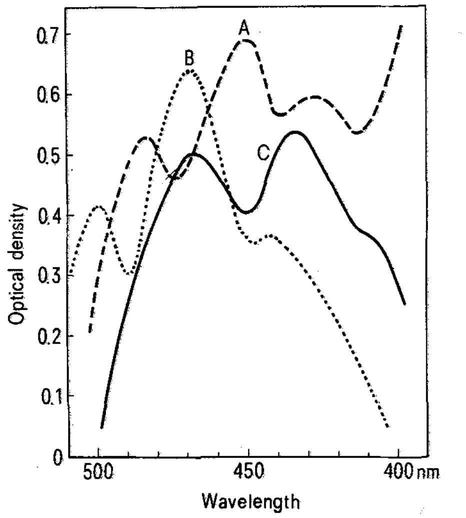
Natural populations of most species of the Polychaete genus *Ophryotrocha* are remarkably uniform with regard to externally visible characteristics. Until now, only a transpecific polymorphism regarding a yellow or white egg coloration has been reported by Akesson[1] in *O. diadema* and by Sella[2] in a population of *O. puerilis siberti* from Roscoff. In *O. diadema,* as in *O. p. siberti,* this polymorphism consists of the presence or absence of yellow pigmentation in both eggs and the animals' body walls. When animals are fed with spinach or nettle, the difference between the 2 forms is very clear. Pigment accumulates first in cells of the gut wall and later in nurse cells and oocytes.

*Genetic analysis of the polymorphism.* Crosses were performed with animals from white and yellow egg stocks, obtained through the courtesy of Prof. Akesson. Animals were reared at constant temperature (20 °C) in vessels containing 10 ml of filtered sea water with density of 0.024 g/l and were fed with parboiled spinach. Food was always in excess and the sea water was changed once a week. Because *O. diadema* is a contemporary hermaphrodite, single mating pairs were made only when both mates showed oocytes, in order to be sure of their phenotype.

F$_1$ progeny of crosses of yellow with white egg individuals always produced yellow egg individuals. Results of 18 mat-

Observed and expected phenotypic ratios in the offspring of $F_2$ (A), backcrosses (B) and progeny test (C). Expected values for $\chi^2$-tests were calculated on the basis of 3:1, 1:1 and 2:1 ratios respectively. In (C) no heterogenity test was carried out because data came from a random sample of $F_2$ yellow egg individuals

| | A | | | B | | | C | | |
|---|---|---|---|---|---|---|---|---|---|
| | Offspring from 18 $Yy \times Yy$ crosses adults phenotypes | | | Offspring from 12 $Yy \times yy$ crosses adults phenotypes | | | Genotypes of $F_2$ yellow egg individuals | | |
| | Yellow eggs | | White eggs | Yellow eggs | | White eggs | $Yy$ | | $YY$ |
| Observed | 708 | | 242 | 525 | | 469 | 384 | | 174 |
| Expected | 712.5 | | 237.5 | 497 | | 497 | 372 | | 186 |
| Deviations | −4.5 | | 4.5 | 28 | | −28 | 12 | | −12 |
| | $X^2$ | d.f. | P | $X^2$ | d.f. | P | $X^2$ | d.f. | P |
| Total | 14.31 | 18 | 0.71 | 19.99 | 12 | 0.07 | | | |
| Pooled data | 0.11 | 1 | 0.74 | 3.15 | 1 | 0.07 | 1.16 | 1 | 0.28 |
| Heterogeneity | 14.2 | 17 | 0.65 | 16.85 | 11 | 0.11 | | | |



Absorption spectra of yellow egg pigment, in benzene (A), $CS_2$ (B) and in ethyl ether (C).

ings between $F_1$ individuals and 12 backcrosses between $F_1$ individuals and white egg individuals are reported in the table (A, B). Expected phenotypic ratios of offspring were based on the hypotheses that egg color is under the control of a pair of alleles at a single locus and that the allele responsible for yellow color (*Y*) is dominant over the allele responsible for white (*y*). Even if from the pooled data a slight excess of *Yy* individuals was observed, none of the tests of goodness-of-fit was significant. The large heterogeneity $\chi^2$-value of backcrosses originates from 2 progenies which, for unknown reasons, departed significantly from expectations. Finally, a progeny test was performed on a random sample of 558 $F_2$ individuals with a yellow egg phenotype to determine whether the ratio of *Yy* to *YY* individuals deviated from the expected 2:1 ratio (table, C). On the whole, the data support the hypothesis that the presence or absence of yellow pigment can be considered as a simple mendelian character and that more complex genetic models can be rejected.

It is noteworthy that heterozygous eggs and embryos can be yellow or white according to the mother phenotype. Likewise, both *yy* and *Yy* embryos derived from a backcross will have the same color, depending on the mother color. This maternal influence on the embryo phenotype generally disappears at the beginning of the free-swimming larval stage.

*Pigment identification.* For the determination of the pigment composition of eggs, yellow eggs spawned by *YY* couples were collected and carefully cleaned, under a stereoscopic microscope, of all excrements from parent worms. Pigments of eggs and spinach leaves were extracted with acetone and exane, according to the Veerman method[3]. The separation and identification of the pigments was performed by thin-layer co-chromatography on silicagel G and aluminium oxide plates (Merck, Darmstadt). A mixture of acetone-exane (1:4) was used as eluent. Absorption spectra were recorded on a Pre-Unicam SP8-100 spectrophotometer.

By its behavior on co-chromatography with samples of known carotenoids, extracted from nettle leaves and purified according to Kuhn et al.[4] and by its absorption spectra (fig.), the egg pigment was identified as lutein.

*Discussion and conclusions.* Co-chromatographic analysis of pigments present in spinach leaves and in *O. diadema* yellow eggs shows that of the many carotenoids present in food, lutein is the only one to be absorbed by these Polychaetes. This result seems to indicate that the recessive *y* allele of the color-determining locus acts by preventing the normal uptake of lutein from food. Some other examples of invertebrates exercising a qualitative selection on dietary carotenoids are known[5-8].

A probable involvement of carotenoids and carotenoproteins in reproduction and development has been indicated in a number of studies[9-11], although conclusive proof for their actual function has not yet been obtained. At least as far as embryonic development of *O. diadema* is concerned, lutein does not seem to be of vital importance. In fact, in the present experimental conditions, no statistically significant differences were observed in developmental rate and embryonic viability between *Yy* embryos developing from white oocytes fertilized by sperms bearing the *Y* allele and *Yy* embryos developing from yellow oocytes fertilized by sperms bearing the *y* allele.

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