Chromosome Analysis of Apple Snail Pomacea canaliculata

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Chromosome Analysis of Apple Snail *Pomacea canaliculata*

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Summary

Chromosomes of three morphologically differentiated strains of *Pomacea canaliculata* were studied using the dropping-air drying method of gill tissue cell suspension. Diploid chromosome number is 2n=28, and the karyotype is composed by 20 M+6 SM+2 ST in female, while it is 19 M+7 SM+2 ST in male, indicating the existance of a male heterogamety (XY).

The mitotic index in gill tissue of adult animals is also unexpectedly high in apple snail.

The apple snail *Pomacea canaliculata* (Cl. Gastropoda: F. Pilidae), a fresh water snail introduced recently in Japan from South America as a culture alternative for food (1), but it turned out to be harmful to water plant cultivars, and the most affected crops are rice and lotus.

This species, on the other side, is suitable to become a model organism for genetic research and breeding experiments (2), showing a short life cycle, high number of eggs with a size of 2 mm diameter, high hatching rate (up to 50%) after two weeks, and easy rearing conditions.

Pomacea canaliculata populations show different phenotypic colormorphs and shell shapes, originating the strains A, B and C. The B strain is dark colored with a light color band, and is considered to be the typical apple snail. The C strain is slightly different from the B strain because of the lack of the light colored band. The strain A, instead, is completely different, showing a light shell color without any color band. These strains are also different in their salinity tolerance (3) and this result leads to the conclusion that the strains have different genotypes.

A first attempt to clarify the genetic differences was made by Fujio and von Brand (2), analyzing the LAP isozymes is apple snail.

More biological and genetic information is not available for this species. Patterson (4) in his review about "Chromosomes of Molluscs" did not include

Pomacea canaliculata.

The purpose of the present study is to determine the diploid chromosome number and the karyotype for each strain A, B and C, and the mitotic index correlated to growth for the normal type, or strain B.

Materials and Methods

The diploid chromosome number of the apple snail *Pomacea canaliculata* was determined using individuals from the strains A, B and C, These were weighted and sexed (Fig. 1) before the chromosome preparation was performed.

The number of individuals taken from the different strains were : 15 from A, 25 from B, and 10 from C. Animals belonging to each strain were kept together, but separately from the other strains to avoid confusion.

The body weight data of strain B were analyzed, and their standard deviation calculated. These results were related to age (days after hatch).

The chromosome preparation technique used is as follows : the whole animal was kept in a 0.1% colchicine solution during four hours. Then the removed gill was chopped in physiological saline (0.9% NaCl), and a cell suspension produced adding a hypotonic solution of KCl (0.075 M) pipetting slowly. This mixture was centrifugated during five minutes at 800 rpm, discarding the supernatant. This was repeated twice, adding always KCl solution. Then the mixture is kept between 40–80 minutes in an hypotonic treatment. After this period, only half of the supernatant is discarded. The fixation is performed using the same volume

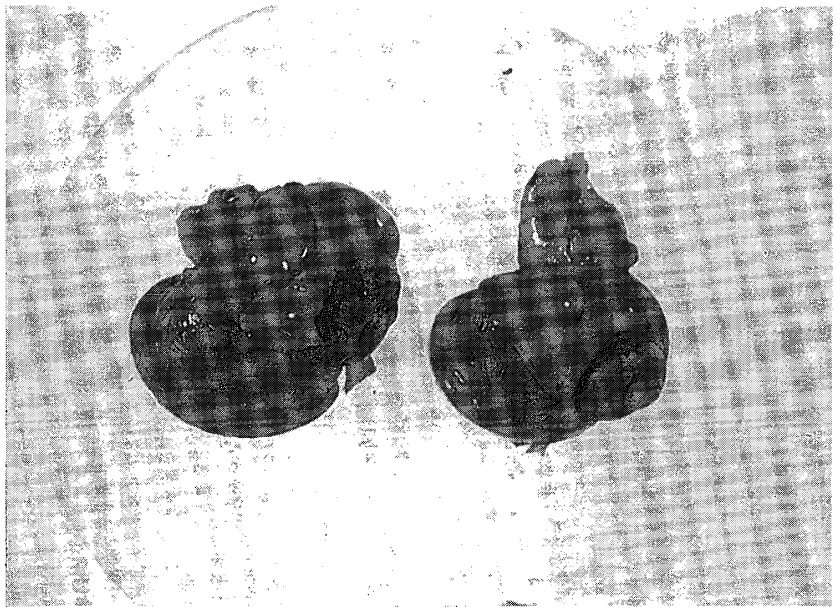

FIG. 1. Anatomical sex differentiation in *Pomacea canaliculata*. Female (left) shows ovary and male (right) a penis.

of the remaining liquid of Carnoy solution (100%), centrifugating again for 5 minutes at 800 rpm, repeating this procedure 4 times, mixing the precipitation thoroughly each time, and adding always Carnoy (100%) after discarding the supernatant. The cells will be resuspended using Carnoy (100%). Afterwards, with a pipette, cell suspension was taken and dropped on a glass slide, letting it air dry. To stain, Giemsa solution was used for 30～40 minutes. The slides were checked using an Olympus Microscope (BH-2), photographing the best plates.

To determine the diploid chromosome number, the number of metaphasic plates scored were 82 for strain A, 77 for strain B, and 44 for strain C. The male and female data were recorded separately for each strain. The most representative metaphasic plates were used to prepare the diploid karyotypes, and determine the arm ratios of the chromosomes for male and female apple snails belonging to the different strains.

The mitotic index for strain B was calculated counting 5,000 nuclei at random, and taking the percentage of nuclei undergoing mitosis over the total number (Fig.2).

Results

The diploid chromosome number was counted at mitotic metaphase plates in gill tissue of apple snail, *Pomacea canaliculata*, and the results are shown in Table 1 for strain A, B and C. The diploid number of chromosomes for the three strains

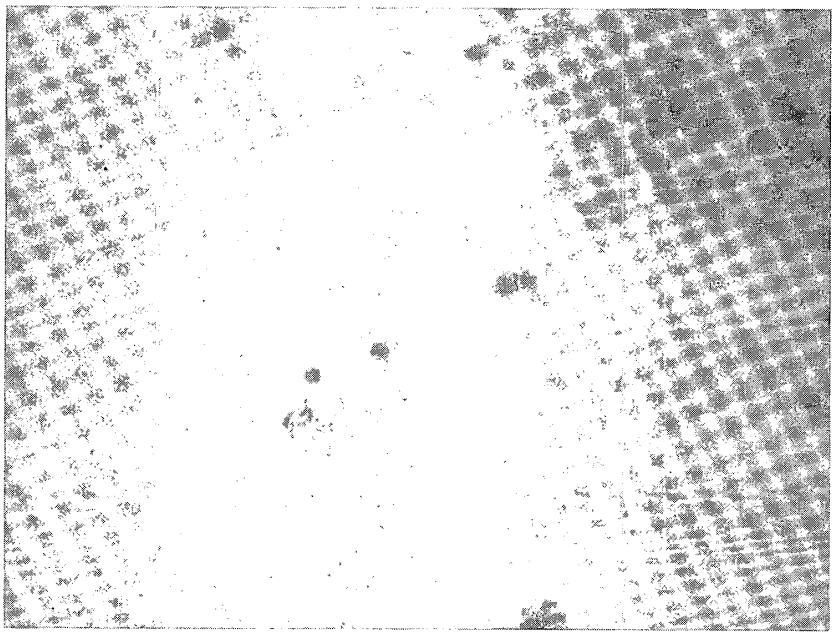

FIG. 2. Preparation for the determination of mitotic index, showing nuclei undergoing mitosis and others not.

TABLE 1. *Chromosome Number of the A, B, and C Strains in the Apple Snail*

	Strain		
	A	B	C
No. Specimens	15	25	10
Plates scored	82	77	44
No. of chromosomes			
2n=19	0	0	0
20	1	0	0
21	0	0	0
22	0	0	0
23	0	0	0
24	1	1	0
25	1	0	0
26	3	0	1
27	3	5	3
28	72	60	40
29	0	1	0
30	1	0	0
31	0	0	0

was 2n=28.

The arm ratio of metaphase chromosomes obtained from gill tissue of female and male animals is shown in Table 2. The sex determination was made by the anatomic differences (Fig. 1) of adult animals, before the processing for chromosomes was initiated.

The female karyotype is composed by 10 metacentric (M) pairs, 3 submetacentric (SM) pairs, and 1 subtelocentric (ST) pair in each of the three strains. The male karyotype, instead, showed 9 M pairs+1 M chromosome, 3 SM pairs+1 SM chromosome, and 1 ST pair in each studied strain. The karyotypes for female and male are shown in Fig. 3 (strain A), Fig. 4 (strain B) and Fig. 5 (strain C),

TABLE 2. *Arm Ratio of Metaphase Chromosomes in the Gill Cells of Three Strains of the Apple Snail*

Arm ratio	A		B		C		Type
	Female	Male	Female	Male	Female	Male	
1.0-1.4	20	19	20	19	20	19	M
1.5-2.0	6	7	6	7	6	7	SM
3.5-5.0	2	2	2	2	2	2	ST

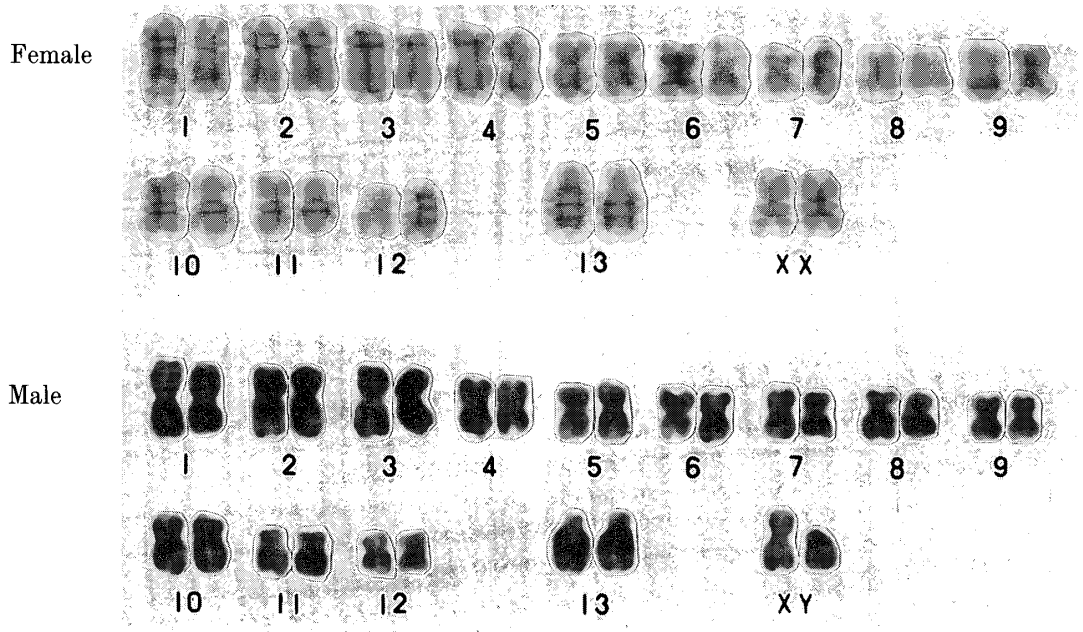

FIG. 3. Karyotype of female and male animals belonging to strain A.

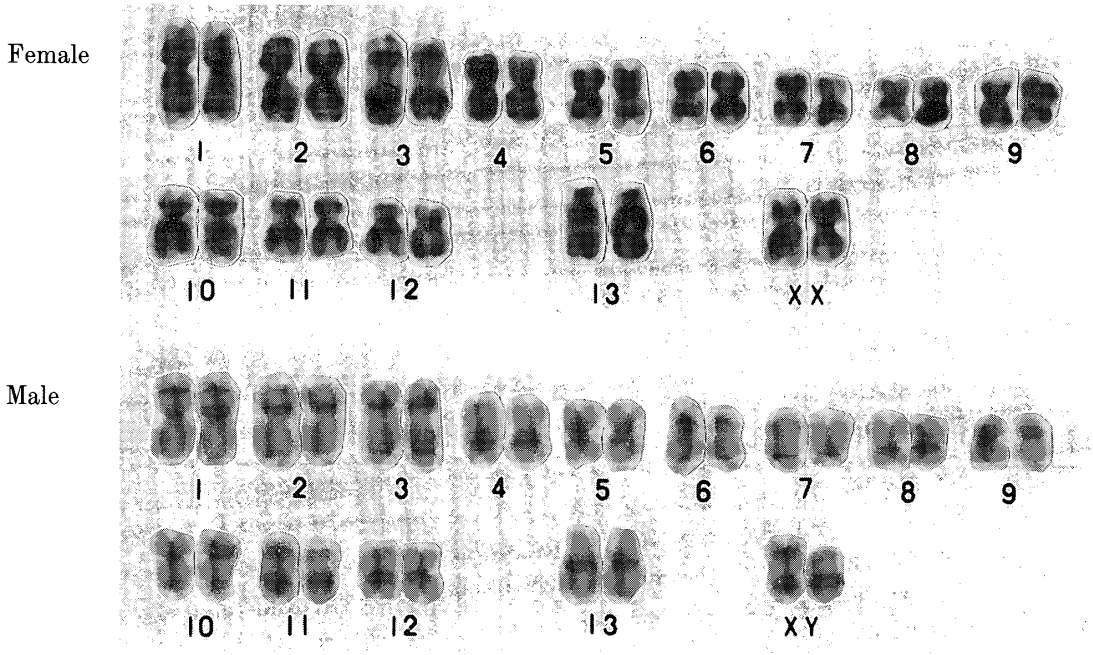

FIG. 4. Karyotype of female and male animals belonging to strain B.

where the existance of one pair male heterosomes (X and Y), and female homologous X chromosomes was determined.

The two heterosomes, X and Y, are morphologically different, being X a metacentric chromosome and Y a submetacentric one and smaller in size.

The mitotic metaphase plates obtained for male and female belonging to each

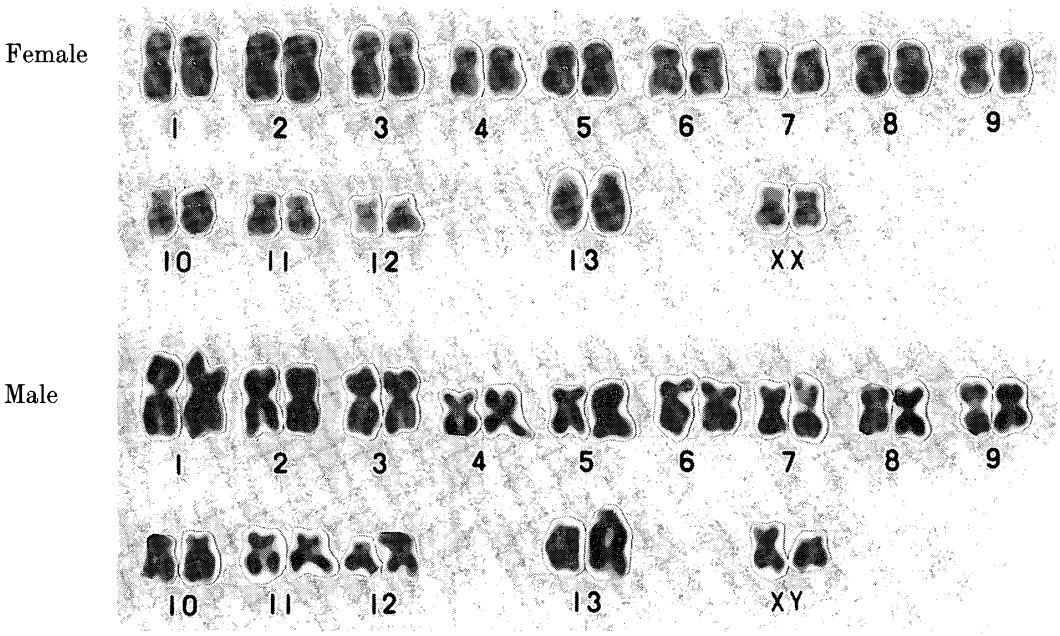

FIG. 5. Karyotype of female and male animals belonging to strain C.

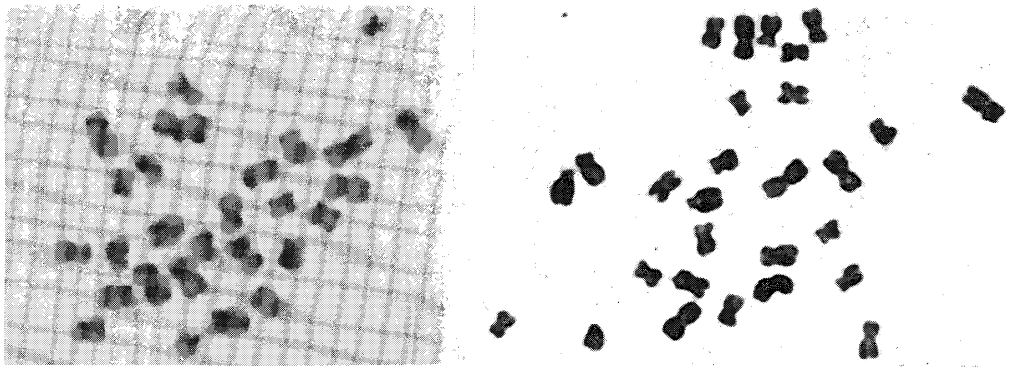

FIG. 6. Metaphasic plates for female (left) and male (right) apple snail belonging to strain A.

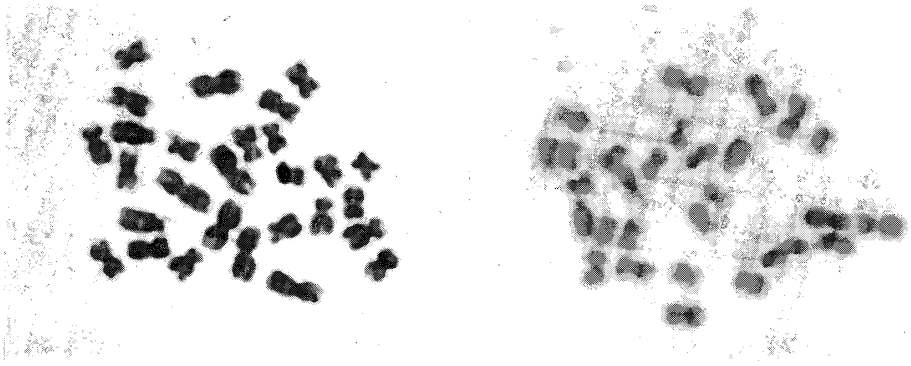

FIG. 7. Metaphasic plates for female (left) and male (right) apple snail belonging to strain B.

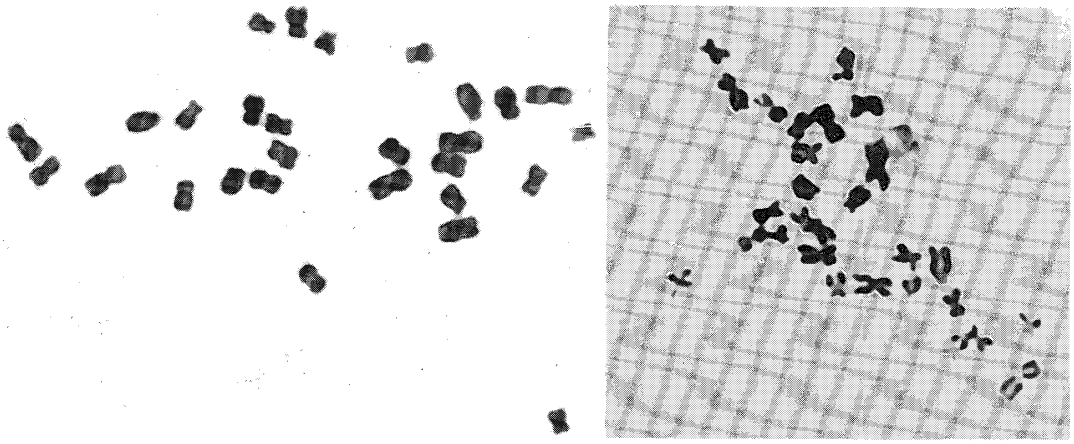

FIG. 8. Metaphasic plates for female (left) and male (right) apple snail belonging to strain C.

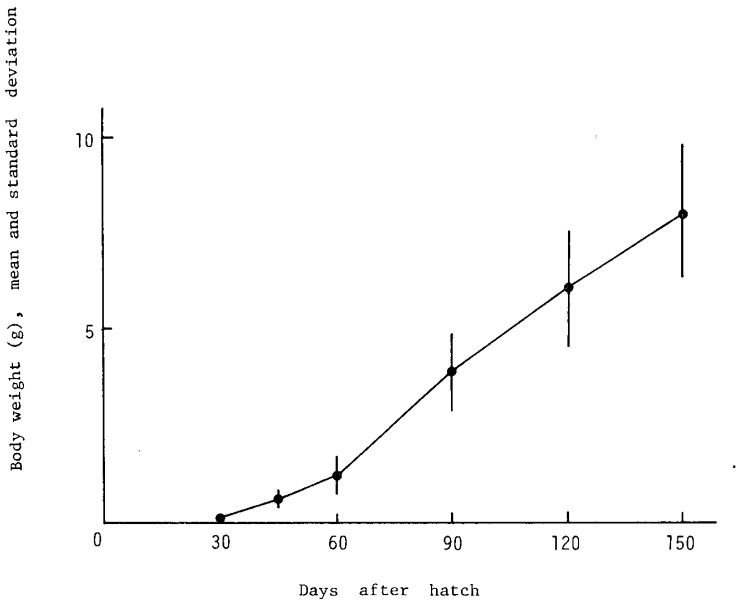

FIG. 9. Growth curve, after hatch in apple snail.

strain are shown in Fig. 6 (strain A), Fig. 7 (strain B) and Fig. 8 (strain C).

The growth curve, after hatch, of the B strain of *Pomacea canaliculata* was determined using body weight (g) and age (days after hatch) data (Fig. 9). The correlation between body weight (g) and mitotic index (%) was calculated, and is shown in Fig. 10, being the mitotic index higher as smaller as the body weight is.

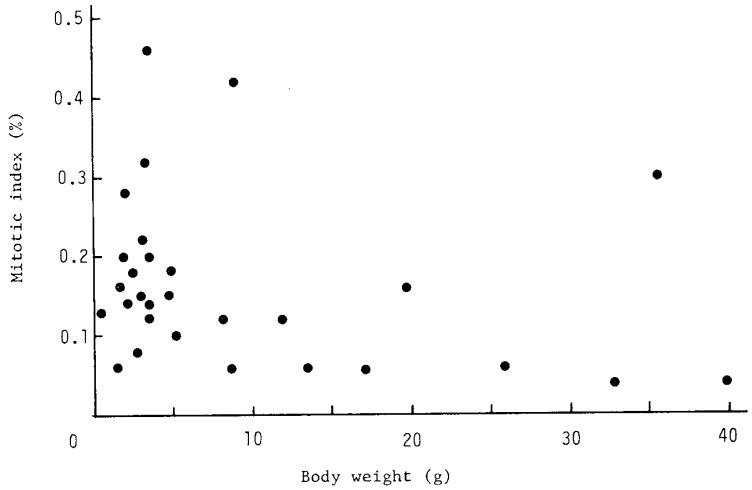

FIG. 10. Mitotic index related to growth stage in apple snail.

Discussion

The fast growth, easy culture and existance of different strains makes the apple snail, *Pomacea canaliculata* an appropriate experimental organism, not only for Genetics, but also for other research areas in Fishery Biology.

The existance of anatomical and chromosomal sex differentition is also a helpful tool in the performance of experiments.

Our karyotype data show the existance of 20 M+6 SM+2 ST in female, and 19 M+7 SM+2 ST in male apple snails, leading these results to the conclusion of the existance of a clear male heterogamety (XY), known also as genotypic sex determination of sex determining mechanisms (6). A similar finding for another molluscan species, *Tulotoma angulata*, was described by Inaba (5), being the chromosome number 2n=26, where the female had 6 M+16 SM+4 ST, and the male 7 M+15 SM+4 ST, indicating also male heterogamety (XY). The chromosomic sex determination is not frequent, like it has been described in fishes (7). Many of these animals show instead functional or sequential hermaphroditism, underlying them a physiological sex determination not seen on chromosomal level. The existance of heterogamety opens a wide range of research possibilities, being the search of sex related genetic markers necessary to determine an inheritance model, useful in molluscan biology.

Analyzing our findings of the diploid chromosome number of individuals of apple snail belonging to the strains A, B and C, not showing karyological differences between each group, arises the question about what type of mechanism is responsible for the phenotypic differences. The environmental influence alone is not able to provoke it, being the results of Kijima *et al.* (3) about differential salinity tolerance, shown by each of the colormorphs, a strong hint to look more

closely at their genotypes using electrophoretic markers and breeding techniques.

At last, the high mitotic index observed in adult gill tissue is unusual in molluscs, being a little higher the one observed in fresh water animals than in marine organisms.

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