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Non-reductional Spermatozoa in Three Shell Color Types of the Freshwater Clam *Corbicula fluminea* in Taiwan

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ABSTRACT—We found three distinct shell color types in samples of the freshwater clam *Corbicula fluminea* Müller collected at Hou Don, Keelung, Taiwan. DNA microfluorometric analysis revealed that these three types consisted of both diploids and triploids. DNA microfluorometry on sperm and somatic cells showed that both diploid and triploid produced non-reductional spermatozoa. These characteristics are similar to triploid *C. leana* Prime sampled in Japan. These findings suggest that *Corbicula fluminea* at different ploidy levels may be reproducing by androgenesis as already shown in *C. leana* from Japan.

INTRODUCTION

The freshwater clams *Corbicula leana* and *Corbicula fluminea* are hermaphroditic and brood their larvae (Miyazaki, 1936; Kraemer and Galloway, 1986). In both species one clam in isolation can reproduce. Reproduction by self-fertilization has been suggested (Ikematsu and Yamane, 1977; Kraemer, 1978; Britton and Morton, 1986). Both species show little allozyme polymorphism (Hills and Patton, 1981; Sakai *et al*., 1994) . This monogenic condition had been explained because of self-fertilization (McLeod and Sailstad, 1980) or gynogenetic development (Okamoto and Arimoto, 1986).

C. leana in Japan was found to be triploid and *C. fluminea* in Taiwan was diploid and both species produce non reductional spermatozoa (Okamoto and Arimoto, 1986; Komaru *et al*., 1997). We have also shown cytological evidence of androgenetic development of triploid *C. leana* in Japan (Komaru *et al*.,1998). Production of non-reductional spermatozoa in *C. fluminea* suggest unusual reproduction such as selfing androgenesis.

According to Morton (1987) and Chen *et al.* (1995) the shell of *C. fluminea* is highly polymorphic. Morton concluded that the polymorphism in shell color in Hong Kong was a result of environmental induction. Morton (1986) also suggested that *C. leana* is a synonym of *C. fluminea* because these two species have similar reproductive characters. The taxonomy in the *Corbicula* is confused due to the polymorphism of the morphology and color of the shell. The taxonomy in this genus should not be based on the shell character only.

We found three clearly different shell color types collected in the same location in Taiwan. To solve the confusion in taxonomy of the *Corbicula*, the mode of reproduction requires examination. As a preliminary study, we compared the DNA content of spermatozoa and somatic cells of these types to determine how these three types reproduce.

MATERIALS AND METHODS

Animals

The *Corbicula fluminea* samples were collected at Hou-Don (25°04'40N, 121°49'00E), Keelung River, Taiwan in June, 1996. Three color types occurred at the same sampling site. The clams were transferred to the National Research Institute of Aquaculture, Nansei, Mie, Japan and kept in an aquarium until analysis. The triploid *C. leana* sample was collected at Meiwacho, Mie Prefecture, Japan.

DNA Microfluorometry of somatic cells and spermatozoa

To estimate the ploidy levels, cells from mantle tissue were isolated in distilled water by cutting the tissues with scissors. Cell suspensions were placed on a slide glass. Two or three samples of *C. fluminea* and triploid cells from one *C. leana* as a standard were placed on the same slide and the fluorescence intensity compared.

Spermatozoa from the gonad and somatic cells from the mantle of the same animals were also placed on the slide using the same procedure. The slides were air dried and fixed with 70% ethanol.

Slides were stained with DAPI staining solution (Hamada and Fujita, 1983) containing 50 ng/ml DAPI and 10 mM 2-mercaptoethylamine hydrochloride in Tris buffer (10 mM Tris, 10 mM EDTA-2Na, 100 mM NaCl, pH 7.2) for at least 30 min and the relative DNA content were measured by microfluorometry (Komaru *et al*., 1988). Nuclei stained with DAPI solution were excited by UV light (365 nm) with an Olympus fluorescence microscope BHS-RFK. The optical conditions are described in Komaru *et al.* (1988). Fluorescence intensity per nucleus was measured with a Nikon P1 photometer.

RESULTS

Shell color

Fig. 1 shows the three color types of the shells collected at Hou Don, Taiwan. Type I: External surface is yellowish brown. Inside is white, but the umbone area is light orange color. This type is characterized by a purple flash along the

Fig. 1. External and internal views of the three type of *Corbicula fluminea* collected in Hou Don, Taiwan. (Left; type I, middle; type II, right; type III) Scale: 10 mm.

anterior and posterior lateral teeth. Type II: External surface is yellowish brown. Inside of shell is white. Type III: External surface is yellowish brown. Inside of shell is deep purple.

Twenty clams sampled consisted of twelve clams of type I, six clams of type II, and two clams of type III.

DNA content of somatic cells

Table 1 shows the mean fluorescence intensity (relative DNA content) of twenty cells from *C. fluminea* and triploid cells. The twenty individuals from Taiwan included eleven diploids and nine triploids. Shell color type I and type II included both diploids and triploids. The two clams of Type III were diploids.

DNA content of sperm and somatic cells

The DNA content of sperm and somatic cells is shown in Table 2. The relative DNA content of spermatozoa was almost identical to those in the somatic cells. Five diploids and seven triploids produced non-reductional spermatozoa. In the other eight clams we could not collect the spermatozoa.

The sensitivity of the photometer was not exactly the same among the samples from individuals in Table 2, so the triploid somatic cells (See 3n-5 and -6 in Table 2) show different mean values.

Table 1. Ploidy estimation by DNA microfluorometry of the three shell colour types (I-III) *Corbicula fluminea* in Keelung, Taiwan

No.	Mean (S.D.) of sample[a]	Mean (S.D.) of 3n standard[b]	Ratio[c]	Ploidy	Shell colour type
1	243.5 (10.7)	369.2 (13.4)	0.66	2n	I
2	223.9 (10.3)	369.2 (13.4)	0.61	2n	I
3	226.1 (11.7)	369.2 (13.4)	0.61	2n	I
4	229.9 (8.9)	341.5 (15.9)	0.67	2n	I
5	303.3 (13.3)	321.3 (12.0)	0.94	3n	I
6	214.8 (8.5)	321.3 (12.0)	0.67	2n	I
7	349.2 (12.7)	363.2 (11.4)	0.96	3n	I
8	337.7 (11.0)	345.8 (15.2)	0.98	3n	I
9	328.8 (12.8)	345.8 (15.2)	0.95	3n	I
10	326.7 (20.0)	345.8 (15.2)	0.94	3n	I
11	327.6 (16.2)	331.0 (11.5)	0.94	3n	I
12	229.5 (13.4)	331.0 (11.5)	0.69	2n	I
13	229.6 (7.5)	331.0 (11.5)	0.99	3n	II
14	306.6 (10.0)	341.5 (15.9)	0.90	3n	II
15	205.8 (7.0)	325.0 (15.3)	0.63	2n	II
16	220.7 (13.4)	325.0 (15.3)	0.68	2n	II
17	233.1 (12.6)	363.2 (11.4)	0.64	2n	II
18	338.2 (6.2)	363.2 (11.4)	0.93	3n	II
19	211.7 (11.3)	341.5 (15.9)	0.62	2n	III
20	234.6 (12.6)	349.3 (24.2)	0.67	2n	III

[a] Mean of relative DNA content (fluorescence intensity) of 20 cells from mantle tissue in *C. fluminea* from Taiwan.
[b] Mean of DNA content of 20 cells from one triploid clam *C. leana* sampled from Mie, Japan.
[c] Ratio = Mean of sample/Mean of 3n standard.

Table 2. Mean (SD) of relative DNA content of spermatozoa and somatic cells in *Corbicula fluminea*.

No.	Shell color type	Mean of spermatozoa			Mean of somatic cells		
2n-1	I	166.43	(7.11)	n = 48	181.77	(12.68)	n = 48
-2	I	155.30	(21.39)	n = 34	168.93	(2.65)	n = 29
-3	II	162.13	(7.31)	n = 31	161.21	(7.98)	n = 23
-4	II	143.00	(6.48)	n = 30	152.50	(8.18)	n = 26
-5	III	162.28	(10.78)	n = 32	169.46	(12.9)	n = 47
3n-1	I	313.15	(13.87)	n = 13	311.42	(18.08)	n = 38
-2	II	218.57	(9.59)	n = 63	249.33	(13.43)	n = 63
-3	I	298.80	(17.94)	n = 48	309.90	(18.10)	n = 49
-4	I	287.79	(13.32)	n = 54	288.21	(16.46)	n = 46
-5	I	314.48	(15.82)	n = 53	326.12	(18.39)	n = 41
-6	I	170.20	(9.51)	n = 43	175.14	(9.30)	n = 34
-7	II	274.72	(16.03)	n = 57	283.30	(18.99)	n = 55

DISCUSSION

The shell color of *C. fluminea* is highly polymorphic (Morton 1987). Hills and Patton (1981) and Morton (1987) recognized two distinct shell color types. Morton concluded that this polymorphism in shell color was due to environmental differences. Morton (1987) and Tsoi *et al.* (1991) showed no genetic differentiation by allozyme analysis between the two color types for samples from Hong Kong. In the present study three color types were collected at one cite. Because the environment was common, distinct shell color types appear to be derived from genetic differences only.

In the present study diploid and triploid *C. fluminea* from Taiwan produce non-reductional spermatozoa as shown from *C. leana* in Japan (Komaru *et al.*, 1997). *C. leana* reproduces by selfing androgenesis as Komaru *et al.* (1998) cytologically confirmed. *C. leana* and *C. fluminea* have similar reproductive characters such as hermaphroditism, brooding, and production of non-reductional spermatozoa. This suggests that *C. fluminea* in Taiwan may also reproduce by a unique mode such as androgenesis.

It is worth mentioning that type I and II color types included both diploids and triploids. We suggest the possibility that the triploid clams may have arisen from diploid clams with the same shell color type which have co-existed. The three shell color types may correspond to different genetical backgrounds. This hypothesis needs to be examined by a genetic study using molecular markers.

The taxonomy of the *Corbicula* is confused, because they show polymorphism in shell morphology and color (Morton, 1986). Hundreds of species have been described based on the shell character alone. *Corbicula* taxonomy can not be clarified on the sole basis of shell morphology. Ecological, genetic, and physiological studies are also necessary. The biological species concept can not be applied to these self-fertilizing hermaphroditic animals (Mayr and Ashlock, 1991). Further studies on the genetic difference and reproductive modes to solve the species problems in *Corbicula*.

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