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KARYOLOGICAL STUDIES OF THE HYBRID LARVAE OF *HALIOTIS DISVERSICOLOR SUPERTEXTA* FEMALE AND *HALIOTIS DISCUS DISCUS* MALE

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ABSTRACT To determine the genomic composition of the interspecific hybrid between *Haliotis diversicolor supertexta* ♀ and *H. discus discus* ♂ at an early developmental stage, veliger larvae produced from hybrid (SJ-5 and SJ-50) and pure species crosses (SS and JJ) were sampled and analyzed using standard karyological methods and genomic *in situ* hybridization. In hybrid metaphase spreads, chromosomes from both parents were detected, except one metaphase, which showed the *H. diversicolor supertexta* haploid karyotype. The genomic composition of the hybrid was also confirmed through preliminary genomic *in situ* hybridization results. Many more aneuploids and chromosome fragments were found in the hybrids than those in the control pure species crosses, indicating genome instability and chromosome loss in the hybrids. In the hybrid hypodiploid metaphase spreads, two intact sets of *H. diversicolor supertexta* chromosomes and several *H. discus discus* chromosomes were detected by pairing. Spontaneous diploidization of the maternal chromosome set was shown to occur in hybrid larvae, as 2.2% heterogeneous triploid and 17.9% hypodiploids with two intact *H. diversicolor supertexta* chromosome sets for SJ-5. The current findings suggest that uniparental chromosome elimination along with spontaneous diploidization of maternal chromosome sets may be the reason for allogynogenesis production in *H. diversicolor supertexta* × *H. discus discus* hybridization.

KEY WORDS: abalone, interspecific hybridization, karyotype analysis, gynogenesis, *Haliotis diversicolor supertexta*, *Haliotis discus discus*

INTRODUCTION

In genetic improvement programs, interspecific hybridization is a useful tool to combine genes from different species. However, true hybrids are not always produced from interspecific hybridization as a result of cross-incompatibility. It is not uncommon for viable progenies to result from parthenogenesis in fish (Chevassus 1983, Howell et al. 1995). Similar phenomena have also been reported in molluscs (Jiang et al. 1983; Li et al. 1983). In other reported cases, the F1 progeny inherit genes from both parents, but genes from one of the parents are preponderant (e.g., Wan et al. 2004). Allen and Gaffney (1993) considered that the viable uniparental progenies from interspecific hybridization might result from contamination by brood from pure crosses because nonparental bands were found in protein electrophoresis of F1 progeny. Hence, it is necessary to investigate the genomic composition of hybrids in molluscs at early developmental stages to reveal whether and how parthenogenetic individuals are produced from interspecific hybridization.

The small abalone (*Haliotis diversicolor supertexta*) and the Japanese abalone (*H. discus discus*) are 2 economically important species cultured in China. The optimal temperature ranges for their growth are different: 23–28°C for *H. diversicolor supertexta* and 18–24°C for *H. discus discus*. They spawn during different seasons, but there is a period of overlap when the water temperature is between 20°C and 25°C. Ke et al. (2000) carried out interspecific hybridization between *H. diversicolor supertexta* and *H. discus discus,* and produced a viable hybrid F1 from both reciprocal crosses. The preliminary results showed that the viable hybrid F1 from the reciprocal cross was not intermediate, but rather was similar to the female parent. In the current study, karyotypes of hybrid larvae of *H. diversicolor supertexta* × *H. discus discus* were analyzed to reveal their genomic composition and the mechanism by which they become gynogenetic diploid.

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MATERIALS AND METHODS

Broodstock and Crosses

Broodstock used in all crosses were obtained from the Haitian Abalone Farm in Southeast China. The parent abalone *H. diversicolor supertexta* and *H. discus discus* were the domesticated descendants originated from the coast of China and Japan, respectively. Each parent abalone was allotted to a separate basin and was induced to spawn artificially by exposure to air and ultraviolet-irradiated seawater. Eggs from *H. diversicolor supertexta* were collected immediately on spawning and fertilized with sperm from *H. discus discus,* as described by Cai et al. (2006). A sample of eggs was retained unfertilized to survey whether independent cleavage or contamination of sperm from the same species would occur. The fertilized eggs were rinsed with fresh seawater every hour.

Samples with 5% and 50% fertilization success were collected 16 h and 18 h after fertilization, respectively. The samples are denoted as SJ-5 and SJ-50 hereafter. Control samples from the pure crosses of *H. diversicolor supertexta* × *H. diversicolor supertexta* and *H. discus discus* × *H. discus discus* were also sampled and are denoted as SS and JJ.

Chromosome Preparation

Specimens for chromosomal studies were obtained according to the method described by Arai et al. (1982) with little modification. Larvae were reared in seawater containing 0.05% colchicine (Sigma, St. Louis, MO) for 1 h, subjected to hypotonic treatment with 0.075 mol/L KCl for 1 h, and fixed in

a fresh solution of acetic acid:methanol (1:3) for 30 min and repeated for a total of 4 times. After fixation, the cell suspension was dropped on slides, air-dried, and stained with 4% Giemsa solution for 15–20 min.

Chromosome Counts and Karyological Study

Well-spread metaphases were acquired using a Leica DC300 F CCD camera attached to a Leica DC300 F CCD (Leica, Wetzlar, Germany). A total of 64 metaphases, 71 metaphases, 275 metaphases, and 53 metaphases were counted for SS, SJ, SJ-5, and SJ-50, respectively.

The length of the short arm, long arm, and total chromosome length were measured. The relative lengths (percentage of the total length of all chromosomes) and arm index (ratio of long arm to short arm lengths) were determined from these values. Nomenclature for the centromeric position on the chromosome was according to Levan et al. (1964) based on the arm index (metacentric chromosomes, 1.0–1.7; submetacentric, 1.7–3.0; subtelocentric, 3.0–7.0; telocentric, 7.0–∞). Positioning of *H. diversicolor supertexta* and *H. discus discus* chromosomes within the karyotype was based on relative chromosome length and arm index. The karyotype was constructed by first dividing the chromosome pairs into classes on the basis of centromere position, and then arranging the homologous pairs in order of decreasing length within each group.

For both SS and JJ groups, karyotypes and quantitative measurements of chromosomes were done using the best 10 metaphases obtained from each abalone species. The ratio of the total length of metacentric chromosomes to that of the submetacentric chromosomes was calculated for both the parent species. Then, the characteristics of the expected heterozygotic diploid (SJ^E) and the expected heterozygotic triploid (SSJ^E) were speculated based on the comparison between *H. diversicolor supertexta* and *H. discus discus*. For the hybrid cross, karyotypes and quantitative measurements of chromosomes were done in every sampled metaphase spread and then compared with the characteristics of the expected hybrid diploid or triploid to judge their chromosome composition.

Genomic in Situ *Hybridization*

The *in situ* hybridization protocol was carried out according to Fujiwara et al. (1997) with minor modifications. Total genomic DNA of both species was extracted from muscle by a conventional phenol–chloroform method. *H. discus discus* DNA was labeled with digoxigenin-11-dUTP by nick translation (Roche Co., Switzerland). The reaction was adjusted to produce DNA fragments of 150–1,000 bp in size as revealed by a smear in a 3% nondenaturing agarose gel. *H. discus discus* DNA for suppression was fragmented to approximately 180 bp average length (range,50–300 bp) by autoclaving for 1.5 min. Labeled *H. diversicolor supertexta* DNA and 75–100 times unlabeled *H. discus discus* DNA were dissolved in 20 µL deionized 100% formamide, denatured for 10 min at 80°C, and then resuspended in a hybridization mixture containing 10% (w/v) dextran sulfate, 0.27% (w/v) bovine serum albumin, 5× SSC (saline sodium citrate buffer; 1× SSC: 0.15 M NaCl, 0.015M Na-citrate, pH 7.0), and 33% (v/v) formamide.

Chromosome slides pretreated with RNase I were denatured at 72°C for 2 min in 70% formamide; 2× SSC; dehydrated in a 70%, 85%, and 100% ethanol series for 5 min each at 4°C; and incubated with the hybridization mixture for 14–18 h at 37°C in a moist chamber. After hybridization, the slides were washed twice in 50% formamide, 2× SSC for 10 min at 42°C, and 2× and 1× SSC for 15 min at room temperature. The digoxigenin probe was immunodetected using the standard protocol with Fluorescein isothiocyanate conjugated antidigoxigenin (Roche, Basel, Switzerland). The chromosomes were counterstained with 1 µg/mL 4,6-diamidino-2-phenylindole (DAPI, Sigma, St. Louis, MO) in VECTASHIELD (Vector Laboratories, UK). Images of fluorescent-stained chromosomes were acquired using a CCD camera attached to a microscope (DM 4500; Leica, Germany) with an appropriate filter and then processed using FW4000 software (Leica, Wetzlar, Germany).

RESULTS

Difference Between the Karyotypes of H. diversicolor supertexta *and* H. discus discus

In the 64 metaphase spreads for *H. diversicolor supertexta*, the chromosome count ranged from 27–64 per metaphase spread with a mode at 32, representing 76.2% of metaphases (Fig. 1A). The karyotype of *H. diversicolor supertexta* consisted of 16 metacentric (m), 14 submetacentric (sm), and 2 subtelocentric (st) chromosomes (Fig. 2A). In the 71 metaphases counted for *H. discus discus,* the number of bivalents varied from 18–54 (Fig. 1B). The mode of the count distribution was found to be 36, representing 85.7% of the metaphases. The karyotype of *H. discus discus* consisted of 20 m and 16 sm chromosomes (Fig. 2B). The differences between *H. diversicolor supertexta* and *H. discus discus* karyotypes are summarized in Table 1. Chromosome pair 16 of the *H. diversicolor supertexta* karyotype can serve as a marker chromosome pair, as they are subtelocentric (or telocentric in some metaphases) chromosomes and quite different from all chromosome pairs for *H. discus discus*. In addition, the ratio between the total length of metacentric chromosomes and that of the submetacentric chromosomes of *H. diversicolor supertexta* and *H. discus discus* was 1.24 ± 0.04 and 0.98 ± 0.03, respectively. According to the difference between the karyotype of *H. diversicolor supertexta* and that of *H. discus discus*, the number of total chromosomes, the number of each type of chromosome, and the ratio of the total length of metacentric chromosomes and that of the submetacentric chromosomes of the expected heterozygotic diploid (SJ^E) to the expected heterozygotic triploid (SSJ^E) were speculated, as shown in Table 1.

The Chromosome Set of SJ Hybrid Larvae

For hybrid larvae, 275 metaphases and 53 metaphases were analyzed in the SJ-5 and SJ-50 groups, respectively. Both histograms of the chromosome number of SJ-5 and SJ-50 had only 1 peak (Fig. 1C, D). The modal number of chromosomes for the SJ-5 group was 32 with a frequency of 20.6%, and the SJ-50 group was 34 with a frequency of 26.9%. The variation in chromosome number of both hybrid groups was much greater than those for both pure cross-species groups (Fig. 1).

Most of the metaphases in the hybrid groups were aneuploids that included hypodiploids and subdiploids. The hypodiploids, those with more than 34 chromosomes, carried 2 marker chromosomes of *H. diversicolor supertexta*, representing 17.9% in SJ-5 and 5.8% in SJ-50. Figure 3A and B shows 2 examples of

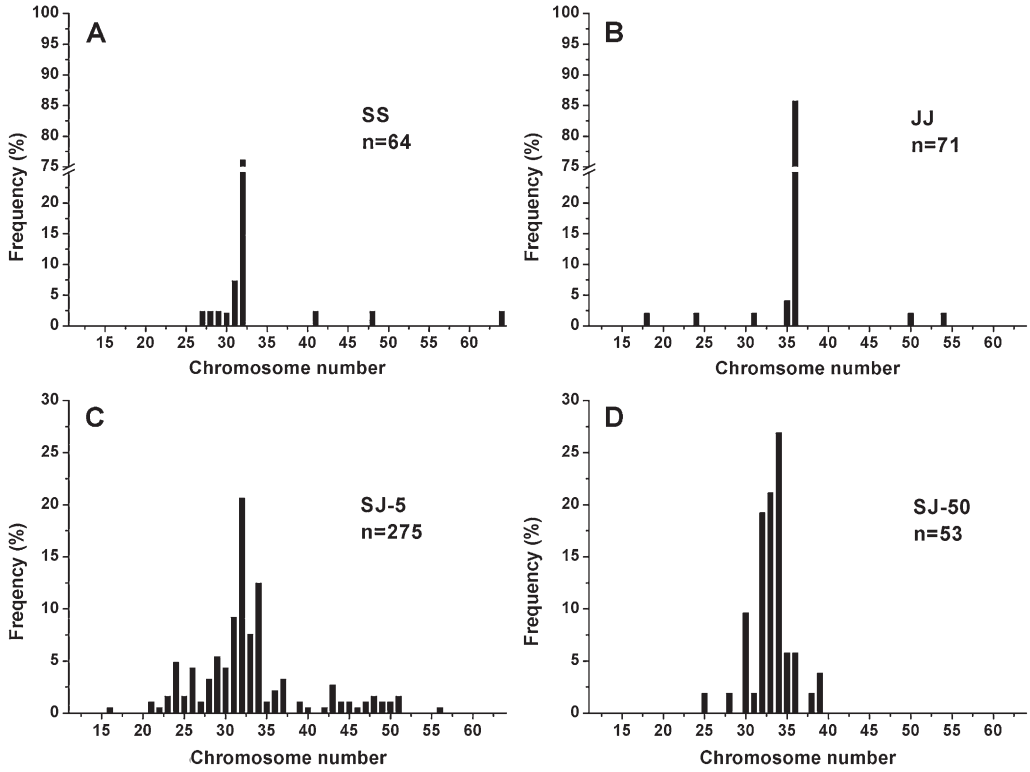

Figure 1. (A–D) Chromosome number distribution for the pure crosses of *H. diversicolor supertexta* (SS; A) and *H. discus discus* (JJ; B), and the interspecific cross between *H. diversicolor supertexta* ♀ and *H. discus discus* ♂ with a fertilization rate of 5% (SJ-5; C) and 50% (SJ-50; D).

hypodiploid metaphase spread and their karyograms. The chromosomes of these 2 metaphases were preliminarily divided into 2 groups by pairing. Group 1 represents chromosomes that may inherit from *H. diversicolor supertexta* and group 2 represents chromosomes that may inherit from *H. discus discus*. The subdiploids, those with less than 34 chromosomes, carried only 1 marker chromosome of *H. diversicolor supertexta,* as shown in Figure 4, which indicates that they contain only one

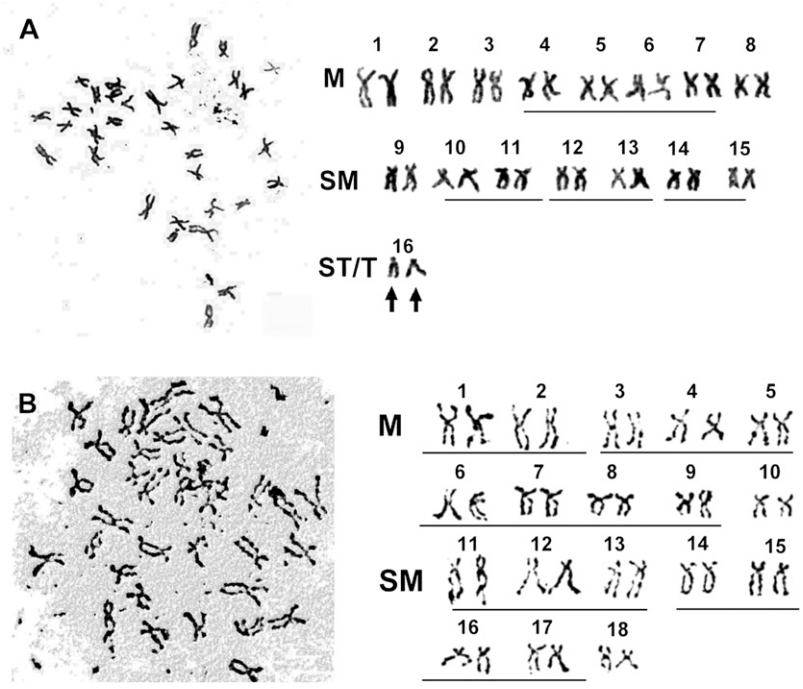

Figure 2. (A, B) Metaphase spreads and karyograms of *H. diversicolor supertexta* (A) *and H. discus discus* (B). Arrows indicate the marker chromosomes of *H. diversicolor*.

TABLE 1.

Comparison between the karyotype of *Haliotis diversicolor supertexta* (SS), *H. discus discus* (JJ), expected heterozygotic diploid (SJ^E), and expected heterozygotic triploid (SSJ^E).

	Index				
Group	CN	m	sm	st	$R_{m/sm}$
SS	32	16	14	2	1.24 ± 0.04
JJ	36	20	16	0	0.98 ± 0.03
SJ^E	34	18	15	1	0.98 – 1.24
SSJ^E	50	26	22	2	0.98 – 1.24

CN, chromosome number; m: metacentric chromosome; $R_{m/sm}$, the ratio between total length of metacentric and submetacentric chromosomes; sm, submetacentric chromosome; st, subtelocentric chromosome.

H. diversicolor supertexta chromosome set, representing 51.9% in SJ-50 and 47.3% in SJ-5.

A small portion of metaphases were euploid, including heterogeneous triploid, heterogeneous diploid, and haploid. About 2.2% metaphases in SJ-5 were found to be heterogeneous triploid containing 2 *H. diversicolor supertexta* chromosome sets and 1 *H. discus discus* chromosome set, as shown in the metaphase spreads and karyograms in Figure 3C and D. The chromosomes of the metaphases were preliminarily divided into 2 groups by pairing: Group 1 represents 2 chromosome sets inherited from *H. diversicolor supertexta* and group 2 represents 1 chromosome set inherited from *H. discus discus*. Heterogeneous diploids consisting of a chromosome set from each parent were found in 12.5% metaphases of SJ-50 and in 26.9% metaphases of SJ-5. Figure 3E shows an example of metaphase spread and its karyogram of a heterozygous diploid. One metaphase in SJ-5 was found to contain only 1 set of chromosomes from *H. diversicolor supertexta* (Fig. 3F). Of the total metaphases analyzed for hybrid larvae in both groups, none had the same karyotype as diploid *H. diversicolor supertexta* or diploid *H. discus discus*. In addition, abundant chromosome fragments were found in the analyzed metaphases in both hybrid groups.

Genomic In Situ *Hybridization*

Figure 5 shows the results of hybridization of total genomic DNA from *H. discus discus* to chromosomes of the SJ hybrid larva (green fluorescence). Several chromosomes and a fragment were uniformly painted green whereas 16 chromosomes remained blue.

DISCUSSION

To detect the parental origin of the chromosomes in the metaphase spread of *H. diversicolor supertexta* × *H. discus discus* hybrid larvae, we determined the karyotypes of *H. diversicolor supertexta* and *H. discus discus,* and confirmed previous reports by Arai et al. (1982, 1988) for these species. The differences between the karyotype of *H. diversicolor supertexta* and *H. discus discus* are summarized in Table 1. Although it is difficult to detect each chromosome's origin in the hybrid metaphases on the basis of only its morphological characteristics through the standard karyological method, we can deduce

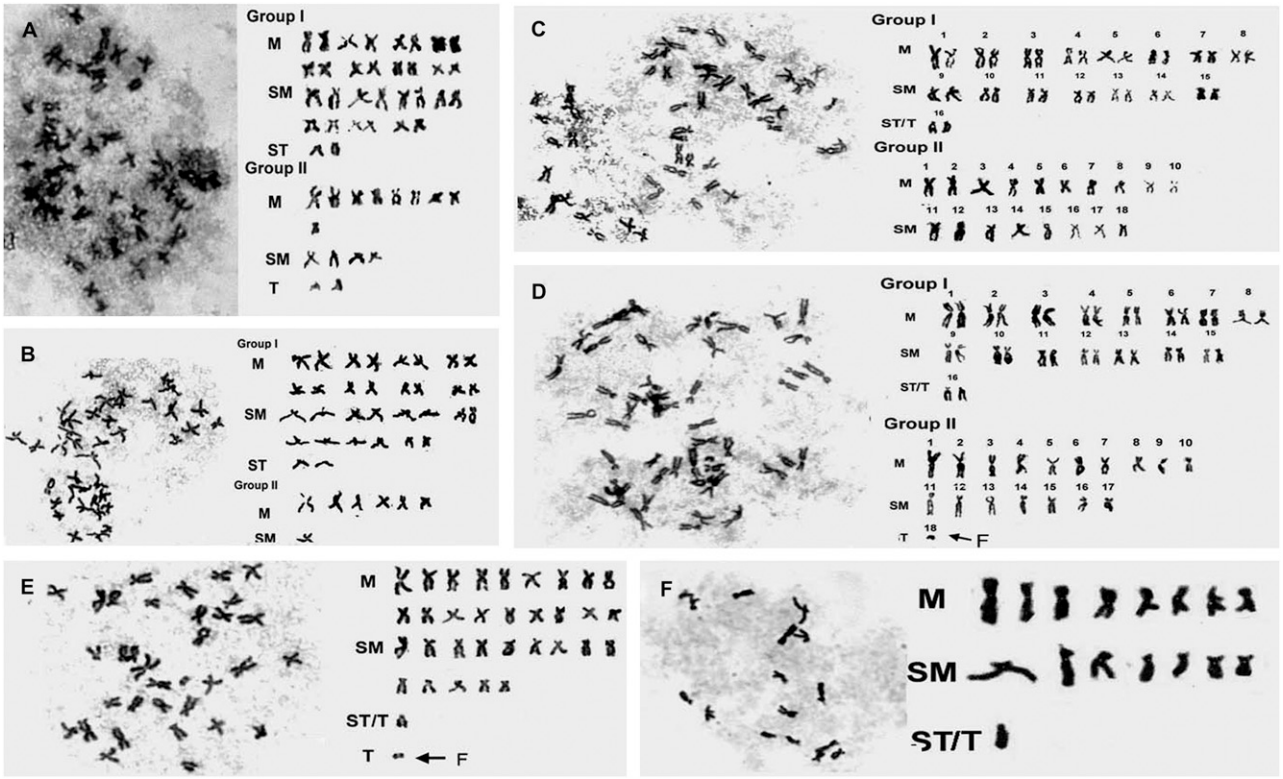

Figure 3. (A–F) Metaphase spreads and karyograms of hypodiploids (A, B), heterogeneous triploid (C, D), heterogeneous diploid (E), and haploid (F) of hybrid larvae between *H. diversicolor* × *H. discus discus*. (F) Chromosome fragment. Group 1 represents the chromosome set inherited from *H. diversicolor supertexta*; group 2 represents the chromosome set inherited from *H. discus discus*.

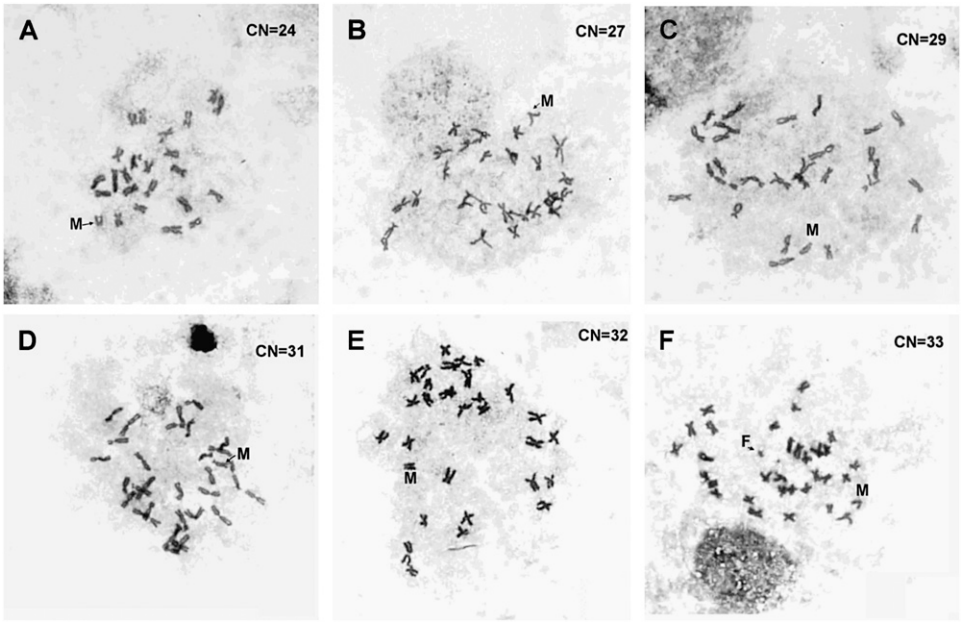

Figure 4. (A–F) Metaphase spreads of subdiploids of hybrid larvae of *H. diversicolor* × *H. discus discus*. CN, chromosome number; F, chromosome fragments; M, marker chromosome of *H. diversicolor*.

the genomic composition of the hybrid on the basis of 4 criteria: (1) the number of *H. diversicolor supertexta* marker chromosomes, (2) chromosome pairing, (3) the number of chromosomes of each type, and (4) the ratio between the total length of metacentric chromosomes and submetacentric chromosomes.

Based on these 4 criteria, we determined that all but one of the analyzed metaphase spreads in the hybrid groups contained the chromosomes from both parents. This was also proved by the genome *in situ* hybridization (GISH) result, which showed the chromosomes from both *H. diversicolor supertexta* and *H. discus discus* with different color during the same metaphase. In molluscs, it is common that the interspecific hybrids during the early development stage contain genetic material from both parents. Jiang et al. (1983) reported that the hybrid embryos of *Pinctada martensii* ♀ × *P. chemnitzi* ♂ contained genomes from both parents by using standard karyological analysis. Wan et al. (2004) proved that the hybrid larvae of *Chlamys farreri* ♀ × *C. nobilis* ♂ contained DNA from both parents by using ISSR. In addition, the cytological studies revealed that the donor sperm incorporated into the egg cytoplasm, developed into the male pronucleus, and merged with the female pronucleus eventually in some interspecific crosses in molluscs, such as *C. farreri* × *Patinopecten yesoensis* (Zhou et al. 2003), *Crassostrea gigas* × *C. rivularis* (Scarpa & Allen 1992), and *H. diversicolor supertexta* ♀ × *H. discus discus* ♂ (Cai et al. 2007).

Heterogeneous triploid and hypodiploid, containing 2 sets of *H. diversicolor supertexta* chromosomes, were found in SJ-5 and SJ-50 with frequencies of 17.9% and 5.8% respectively, which suggests that spontaneous diploidization of the maternal chromosome set (SDM) had occurred in a portion of hybrid larvae. SDM with low frequency has also been reported in other species of molluscs, such as *C. gigas* (Guo et al. 1992), *Mytilus californianus,* and *M. edulis* (Ahmed & Sparks 1970). We also observed spontaneous triploids in the larvae of pure species crosses for *H. diversicolor supertexta* and *H. discus discus* (Fig. 1).

Aneuploids were often encountered in chromosome counts for technical shortcomings in chromosome preparation and handling, for chromosome losses; addition might occur for insufficient or excessive spread, which was also commonly observed in karyological analysis in abalone, such as in *H. planata*, *H. varia,* and *H. diversicolor diversicolor* (Arai et al. 1988), and in *H. fulgens* and *H. rufescens* (Hernandez-Ibarra

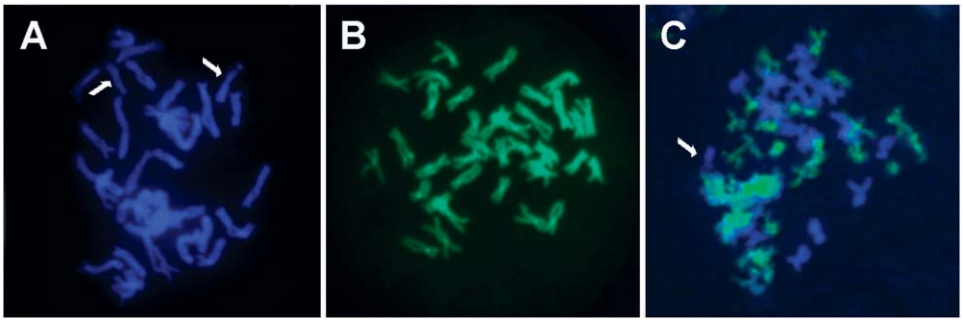

Figure 5. (A–C) Metaphase spreads in *H. diversicolor* (A), *H. discus discus* (B), and their hybrids (C) after GISH with *H. discus discus* genomic DNA. Arrows indicate the marker chromosomes of *H. diversicolor*.

et al. 2004). In the current study, aneuploids were also observed in the pure cross groups: 23.8% for *H. diversicolor supertexta* and 14.3% for *H. discus discus*. However, many more aneuploids and chromosome fragments were observed in the hybrid groups than those in the pure cross groups of both parents, which were not only the result of technical shortcomings in chromosome preparation and handling, but also a result of the instability of the hybrid genome. Some of the chromosomes tend to be eliminated from the genome of *H. diversicolor supertexta* × *H. discus discus* hybrids during development. Uniparental chromosome elimination has been shown in hybrids between masu salmon females and rainbow trout males using the GISH technique (Fujiwara et al. 1997), and in hybrids between grass carp females and common carp males using standard karyotype analysis (Ye et al. 1989). In the current study, based on a detailed investigation of the aneuploid karyotype, we found that the hypodiploid metaphases carried 2 intact sets of *H. diversicolor supertexta* genome and several *H. discus discus* chromosomes. However, more evidence is required to confirm whether the paternal chromosomes were eliminated preferentially from the SJ hybrids.

In the current study, 2 batches of larvae with different fertilization rates were sampled. Although the mode of chromosome number for these 2 batches was different (32 in the SJ-5 group and 34 in the SJ-50 group), the metaphase spreads for both groups contained chromosomes from both parents. The possible reasons for the different modal number of chromosomes for the 2 batches may be sample error or more chromosome elimination in SJ-5, which needs further study.

In conclusion, we propose the possible mechanism of allogynogen production from *H. diversicolor supertexta* × *H. discus discus* interspecific hybridization as follows: *H. discus discus* sperm activate the development of the *H. diversicolor supertexta* eggs (with SDM occurring in some eggs), the genome from the male parent combines with that of the female parent, and *H. discus discus* chromosomes are eliminated preferentially from the resultant composition of the hybrid genome, which was unstable during subsequent mitosis. The SDM hybrid eggs develop into allogynogens after elimination of the *H. discus discus* chromosomes.

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LITERATURE CITED

Ahmed, M. & A. K. Sparks. 1970. Chromosome number, structure and autosomal polymorphism in the marine *Mytilus edulis* and *Mytilus californianus*. *Biol. Bull*. 138:1–13.

Allen, S. K. & P. M. Gaffney. 1993. Genetic confirmation of hybridization between *Crassostrea gigas* Thunberg and *Crassostrea rivularis* (Gould). *Aquaculture* 113:291–300.

Arai, K., H. Tsubaki, Y. Ishitani & K. Fujino. 1982. Chromosomes of *Haliotis discus hannai* Ino and *H. discus* Reeve. *Bull. Jap. Soc. Sci. Fish*. 48:1689–1691.

Arai, K., K. Fujino & M. Kudo. 1988. Karyotype and zymogram differences among three species of the abalones *Haliotis planata, H. varia*, and *H. diversicolor diversicolor*. *Nippon Suisan Gakkaishi* 54:2055–2064.

Cai, M., C. Ke, G. Wang, Z. Wang & Y. Wang. 2006. Influence factors on fertilization rate in laboratory hybridization between *Haliotis diversicolor* and *H. discus discus*. *J. Fishery Sci. China* 13:230–236. [in Chinese with English abstract].

Cai, M., C. Ke, W. You, G. Wang, Z. Wang & Y. Wang. 2007. Cytological studies on the fertilization of the cross between *Haliotis diversicolor* ♀ and *H. discus discus* ♂. *J. Xiamen Univ*. 46: 239–243 [in Chinese with English abstract].

Chevassus, B. 1983. Hybridization in fish. *Aquaculture* 33:245–262.

Fujiwara, A., S. Abe, E. Yamaha, F. Yamazaki & M. C. Yoshida. 1997. Uniparental chromosome elimination in the early embryogenesis of the inviable salmonid hybrids between masu salmon and rainbow trout male. *Chromosoma* 106:44–52.

Guo, X., K. Cooper, W. K. Hershberger & K. K. Chew. 1992. Genetic consequences of blocking polar body I with cytochalasin B in fertilized eggs of the Pacific oyster, *Crassostrea gigas*: I. Ploidy of resultant embryos. *Biol. Bull*. 183:381–386.

Hernandez-Ibarra, N. K., C. Marquez, J. L. Ramirez & A. M. Ibarra. 2004. Comparative karyotypes of two northeastern Pacific abalone species (*Haliotis fulgens* Philippi and *Haliotis rufescens* Swainson). *J. Shellfish Res*. 23:861–866.

Howell, B. R., S. M. Baynes & D. Thompson. 1995. Progress towards the identification of the sex-determining mechanism of the sole, *Solea solea* (L.), by the induction of diploid gynogenesis. *Aquacult. Res*. 26:135–140.

Jiang, W., Y. Wei & G. Li. 1983. Studies on the cultivated interspecific hybridization between pairs of *Pinctada fucata, P. chemnitzi* and *P. maxima* (Mollusca, Bivalvia) II. Observation on the chromosomes of fertilization and hybrids. *Trop. Ocean*. 2:316–320. [in Chinese with English abstract].

Ke, C., Y. Tian, S. Zhou & H. Li. 2000. Preliminary studies on hybridization of three species of abalone. *Mar. Sci*. 24:39–41. [in Chinese with English abstract].

Levan, A., K. Fredga & A. A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. *Heredita* 52:201–220.

Li, G., W. Jiang & Y. Wei. 1983. Studies on the cultivated interspecific hybridizations between pairs of *Pinctada fucata, P. chemnitzi* and *P. maxima* III. Comparative study on zymograms. *Trop. Ocean*. 2:321–328. [in Chinese with English abstract].

Scarpa, J. & S. K. Allen. 1992. Comparative kinetics of meiosis in hybrid crosses of Pacific oyster *Crassostrea gigas* and suminoe oyster *C. rivularis* with the American oyster *C. virginica*. *J. Exp. Zool*. 263: 316–322.

Wan, J., Z. Bao, G. Liu & J. Pan. 2004. Segregation of ISSR markers in hybrid larvae of two scallop species. *High Tech. Lett*. 14:82–87. [in Chinese with English abstract].

Ye, Y., Q. Wu & R. Chen. 1989. Studies on cytology of crosses between grass carp and carp: asynchronization between nucleus and cytoplasm in distant hybridization of fishes. *Acta Hydrobiol. Sinica* 13:234–239. [in Chinese with English abstract].

Zhou, L., A. Yang, Z. Liu, F. Du, L. Zhang & Q. Wang. 2003. Cytological observations on cross fertilization between *Chlamys farreri* (♀) and *Patinopecten yessoensis* (♂) scallops. *Chinese J. Zool*. 38:20–23. [in Chinese with English abstract].