



Abnormal Gametogenesis, Male Dominant Sex Ratio, and Sertoli Cell Morphology in Induced Triploid Mussels, *Mytilus galloprovincialis*

Authors: Kiyomoto, Masato, Komaru, Akira, Scarpa, John, Wada, Katsuhiko T., Danton, Emmanuelle, et al.

Source: Zoological Science, 13(3) : 393-402

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.13.393>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Abnormal Gametogenesis, Male Dominant Sex Ratio, and Sertoli Cell Morphology in Induced Triploid Mussels, *Mytilus galloprovincialis*

Masato Kiyomoto¹, Akira Komaru², John Scarpa³, Katsuhiko T. Wada²,
Emmanuelle Danton⁴ and Masahiko Awaji⁵

¹Department of Biology, Ochanomizu University, Ohtsuka, Bunkyo-ku, Tokyo 112, Japan

²National Research Institute of Aquaculture, Fisheries Agency, Nansei, Watarai, Mie 516-01, Japan

³Harbor Branch Oceanographic Institute, Fort Pierce, Florida 34946, U.S.A.

⁴Laboratoire de Biologie et Biotechnologies Marines, Institut de Biochimie et de Biologie Appliquees, Esplanade de la Paix, Universite de Caen, 14032 Caen cedex, France

⁵National Research Institute of Fisheries Science, 12-4 Fuku-ura, 2-chome, Kanazawa-ku, Yokohama, Kanagawa 236, Japan

ABSTRACT—Gametogenesis of one year-old induced triploid mussels, *Mytilus galloprovincialis*, was examined histologically and compared to sibling diploid mussels. Histological analysis revealed that triploid mussels developed a number of primary spermatocytes that were arrested at prophase I. Late in the reproductive season, triploid mussels produced an extremely small number of spermatozoa (9/10000 μm^2 gonadal section) compared to diploid mussels (1072/10000 μm^2 gonadal section). All triploid mussels were identified as males, whereas the sex ratio of diploid mussels was almost equal (1.12:1.0, male:female), indicating that sex determination for this species may follow a Z:W model. Sertoli cells in triploid mussels were prominent, had an enlarged cytoplasm, and were easily seen using light microscopy. In comparison, Sertoli cells in diploids were thin and could only be seen by electron microscopy. Sertoli cell hypertrophy in triploid mussels probably reflects their role in eliminating abnormal and degenerating germ cells.

INTRODUCTION

Gametogenesis in most triploid bivalves is suppressed, conferring an advantage in growth rate and flesh quality, however, suppression of gametogenesis is differential among and within species (reviewed in Beaumont and Fairbrother, 1991; Guo and Allen, 1994a,b). Induced triploids of the noble scallop *Chlamys nobilis* were completely sterile and produced neither mature oocytes nor spermatozoa (Komaru and Wada, 1988). In the soft shell clam *Mya arenaria* virtually all of the triploids exhibited undeveloped gonads with no mature gametes (Allen *et al.*, 1986). In contrast, normal but reduced gametogenesis occurs in triploid Pacific oyster *Crassostrea gigas* (Akashige, 1990; Allen and Downing, 1990; Guo and Allen, 1994a), triploid Japanese pearl oyster *Pinctada fucata martensii* (Komaru and Wada, 1990), and triploid dwarf surfclam *Mulinia lateralis* (Guo and Allen, 1994b). Gametes from female triploid Japanese pearl oysters and from female and male triploid Pacific oysters were found to be capable of fertilization and development (Komaru and Wada, 1994; Guo and Allen, 1994a).

Another effect of induced triploidy in bivalves is the

alteration of sex ratio or differentiation of gonadal tissue. Triploid soft shell clams were found to be all female, leading the authors to hypothesize an X:autosome balance mechanism for sex determination in this species (Allen *et al.*, 1986), whereas in the dwarf surfclam an X:Y mechanism was proposed from results of induced triploidy and gynogenesis (Guo and Allen, 1994b). Triploid Pacific oysters exhibited an increase in the frequency of hermaphrodites compared to diploids, however the sex ratio in triploids was not significantly different from that in diploids (Allen and Downing, 1990). Consequently, genetically modified organisms can provide important information concerning sex determination mechanisms in bivalves, which has not been established, although hypotheses have been presented.

Triploid animals are also useful for examining basic physiological and endocrinological processes of gametogenesis. An insulin-like peptide has been identified and correlated to the gametogenic cycle in diploid and triploid mussels (Danton *et al.*, 1996). Sertoli cells, a somatic cell of the germinal epithelium, in mollusks have been proposed to be involved in phagocytosis and nutrition of the developing germ cells (Pipe, 1987), maintenance of the blood testis barrier

(Buckland-Nicks and Chia, 1986), and steroid production (Jong-Brink *et al.*, 1981), although some of these functions have not been systematically determined.

In the present study gametogenesis of triploid mussels was examined histologically and compared with that of diploid mussels to quantify differences in gametogenesis, with particular attention on reproductive potential, sex ratio, and Sertoli cell ultrastructure.

MATERIALS AND METHODS

Organism

Triploidy was induced in mussels, *Mytilus galloprovincialis*, using cytochalasin B (CB) to prevent polar body II formation of fertilized eggs (Scarpa *et al.*, 1994). Briefly, natural mussels were collected in January 1992 from Gokasho Bay, Mie Prefecture, Japan and induced to spawn the same day by air exposure and thermal stimulation. Eggs of eight females were pooled and fertilized using sperm pooled from six males. The fertilized egg suspension was divided into two groups; one group, herein referred to as 3N-92, was treated with CB (1mg/l) and the other group served as a control (2N-92). Larvae and juveniles were cultured in tanks following standard procedures (Pechenik *et al.*, 1990). In June 1992, both groups were placed in pearl nets and suspended from a raft in Gokasho Bay.

In January 1993, a second group of triploid and diploid mussels, herein referred to as 3N-93 and 2N-93, respectively, was produced as above using gametes from ten females and nine males. This group was produced to observe if the sex ratio bias was repeatable.

Sampling

From October 1992 to August 1993, ten samples of 10-20 mussels were taken from groups 3N-92 and 2N-92. In October 1993 a single sample of mussels was taken from groups 3N-93 and 2N-93. Individuals for histological analysis by light microscopy were opened slightly, tissues removed for ploidy analysis and then placed whole in

Bouin's fixative. Four hr later shells were removed from each individual and the soft body parts fixed for 48 hr in the same fixative. These samples were then dehydrated in a graded ethanol series and embedded in paraffin. Six μm sections were cut, placed on a glass slide and stained with hematoxylin and eosin. This material was used for stage classification of gametogenesis and stereological analysis (see below).

Ploidy level of each mussel was determined by microfluorometry using gill tissue or hemocytes (Komaru *et al.*, 1988). These tissues were fixed with Carnoy's fixative and stained with the fluorochrome DAPI before analysis (Komaru *et al.*, 1988).

Stage classification of gametogenesis

Terminology for stages of gametogenesis was adapted from Wilson and Seed (1974) and King *et al.* (1989) after partial modification. Gonadal cells were classified into the following five stages: Immature (start of gametogenesis is apparent and small clusters of germinal cells are scattered throughout the connective tissue), Developing (follicles occupy a large part of the mantle, animals restoring their gonad after a partial spawn are included in this category), Ripe (follicles full of oocytes in female and lamellae of ripe spermatozoa packed in male), Spent (follicles begin to collapse and degenerate), and Resorbing-resting animals. However, gametogenesis of triploid mussels was quite different from diploid mussels, necessitating the use of a different set of stages (see results and Fig. 1). During gametogenic analysis the sex of each individual was noted.

Stereological analysis

Gametogenic activity was quantified for triploid and diploid mussels by stereological analysis of gonadal sections according to Weibel *et al.* (1966) as modified by Lowe *et al.* (1982). Analysis was performed on three diploids and three triploids for each sampling. In brief, cells in a section of gonad were examined and assigned to one of the following four categories: pre-meiotic germ cells (spermatogonia and primary spermatocytes), germ cells after meiosis I (secondary spermatocytes, spermatids and spermatozoa), abnormal and degenerating cells, or somatic cells (Sertoli cells and hemocytes).

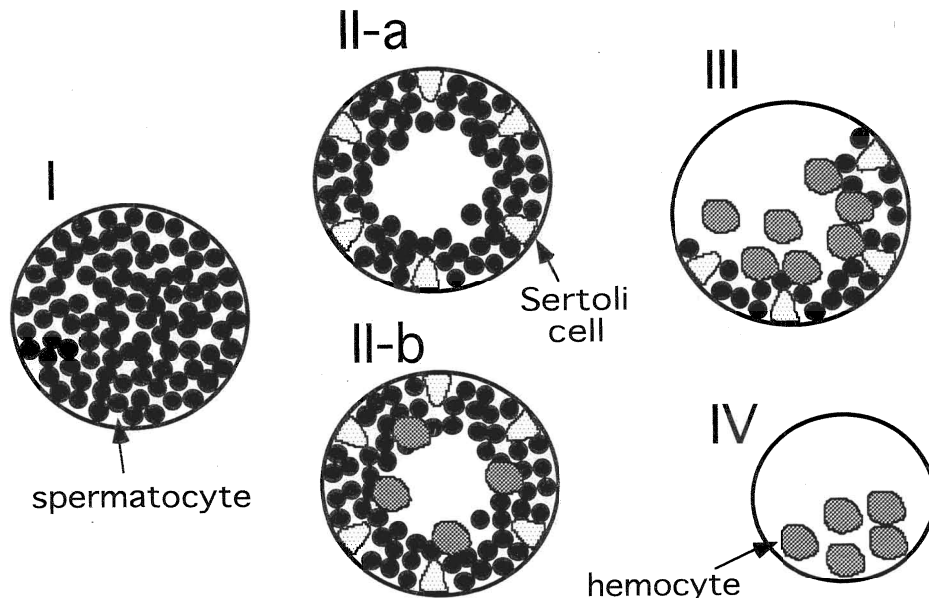


Fig. 1. Gametogenic stages of testis in triploid mussels. Stage I, the testicular acini filled with spermatocytes. Stage II-a, large Sertoli cells on the basement membrane of acini and widened lumen space in central region of acini. Stage II-b, some hemocytes in acini. Stage III, number of germ cells in gonad decreased. Stage IV, germ cells in the gonad resorbed and hemocytes occasionally observed in acini. Spermatogonia on basement membrane and small number of spermatozoa were not included in these figures.

Cell counts were converted to a volume fraction and a two-factor analysis of variance conducted. When ploidy had an overall significant influence on the studied variable, a Sheffe F test was run between diploids and triploids.

Electron microscopy

Preparation of gonadal tissue for transmission electron microscopy followed that of Komaru *et al.* (1994). In brief, each testis was cut into small pieces and prefixed for 12 hr at 4°C with 4% glutaraldehyde in cacodylate buffer (pH 7.5) containing 8% sucrose. Prior to post-fixation, each testis was rinsed with cacodylate buffer. Post-fixation was done with 1% osmium tetroxide in cacodylate buffer for 1 hr at 4°C, followed by dehydration through a graded ethanol series. Each testis was then embedded in Quetol 812. Ultrathin sections were stained with uranyl acetate followed by lead citrate and observed by transmission electron microscopy (JEOL 1200 EX).

RESULTS

Ploidy

Microfluorometric analysis of the 129 mussels sampled from group 3N-92 revealed that 119 (92.2%) were triploid and 10 were diploid. All (n=124) mussels of the control group were diploid. Microfluorometric analysis of 19 mussels sampled from group 3N-93 revealed that all (100%) were triploid and those of group 2N-93 (n=20) were all (100%) diploid.

Sex ratio

The sex ratio of mussels (Table 1) in the triploid group 3N-92 was greatly biased toward males (107 males:0 females; 12 unidentified), whereas the sex ratio of mussels in the diploid group was almost 1:1 (64 males:57 females; 1 hermaphrodite, 2 unidentified). In the four unidentified mussels of group 3N-92 a few oocytes were observed (Fig. 3H), but in these mussels the gonads were resorbed and almost all germ cells disappeared. The second group of triploid mussels (3N-93) sampled in October 1993 exhibited a similar sex ratio bias of males, while the diploid group contained an almost equal proportion of the sexes for the small sample size (Table 1).

Diploid gametogenesis

Gametogenesis had already started in diploid mussels at the time of first sampling (October 1992). Three successive gametogenetic waves were differentiated (Fig. 2). Ripe animals for both sexes were dominant on 1992/12/01, 1993/

01/11, 1993/03/01, and 1993/04/08. Between these dates, gonadal tissue was in the developing stage. In May, almost 50 % of the mussels examined had completely released their gametes (Fig. 2). By the time of the final sampling in August, gonadal degeneration was evident and 40% of mussels were in the resorbing-resting stage.

Triploid gametogenesis

Gametogenesis and gonadal morphology in triploid mussels were altered and therefore different from diploids, necessitating a change in the standard gametogenesis stage nomenclature. Five stages were implemented as illustrated in Fig. 1. The five stages were: I - spermatogonia proliferation: primary spermatocytes were forming and filling the testicular acini (Fig. 3A). II - large Sertoli cells present on basement membrane of acini: degenerating germ cells appeared and the lumen widened in the central region of the acini. Stage II was subdivided into II-a: hemocytes not present (Fig. 3B, C) and II-b: hemocytes present (Fig. 3D, E). III - germ cell number decreasing and replaced by hemocytes (Fig. 3F). IV - germ cells disappearing but hemocytes still present: gonad is resorbing and storage cells (adipogranular cells, A. D. G. cells) are reappearing in mantle (Fig. 3G).

Relative abundance of the different stages in the triploid group is presented in Fig. 2. During the October sampling, stages I and II-a were present, but there was a preponderance of stage I. By early December, there was an increase in the proportion of stage II-a. By late December, stage II-b appeared and increased until April. Stage III appeared during the March sampling, although stage IV was detectable at a level of 10% from early January until March before becoming dominant (67%) during August. No animals could be classified as developing or ripe in the triploid group. A few mature gametes were present during stages II and III, though the number of spermatozoa per 10000 μm^2 was extremely small compared to diploids (Table 2).

Electron microscopic observation revealed synaptonemal complexes in some spermatocytes, whereas other spermatocytes showed condensed chromatin (Fig. 4A, B). These observations suggest that most of spermatocytes in triploid gonads were at the prophase I of meiosis and gametogenesis of triploid mussels was arrested at this stage.

Table 1. Sex ratio

		male	hermaphrodite	female	degenerate	total
born in 1992	diploid (2N-92)	64 51.6%	1 0.8%	57 46.0%	2 2%	124
	triploid (3N-92)	107 89.9%	0	0	12 10.1%	119
born in 1993	diploid (2N-93)	8 40%	0	12 60%	0	20
	triploid (3N-93)	19 100%	0	0	0	19

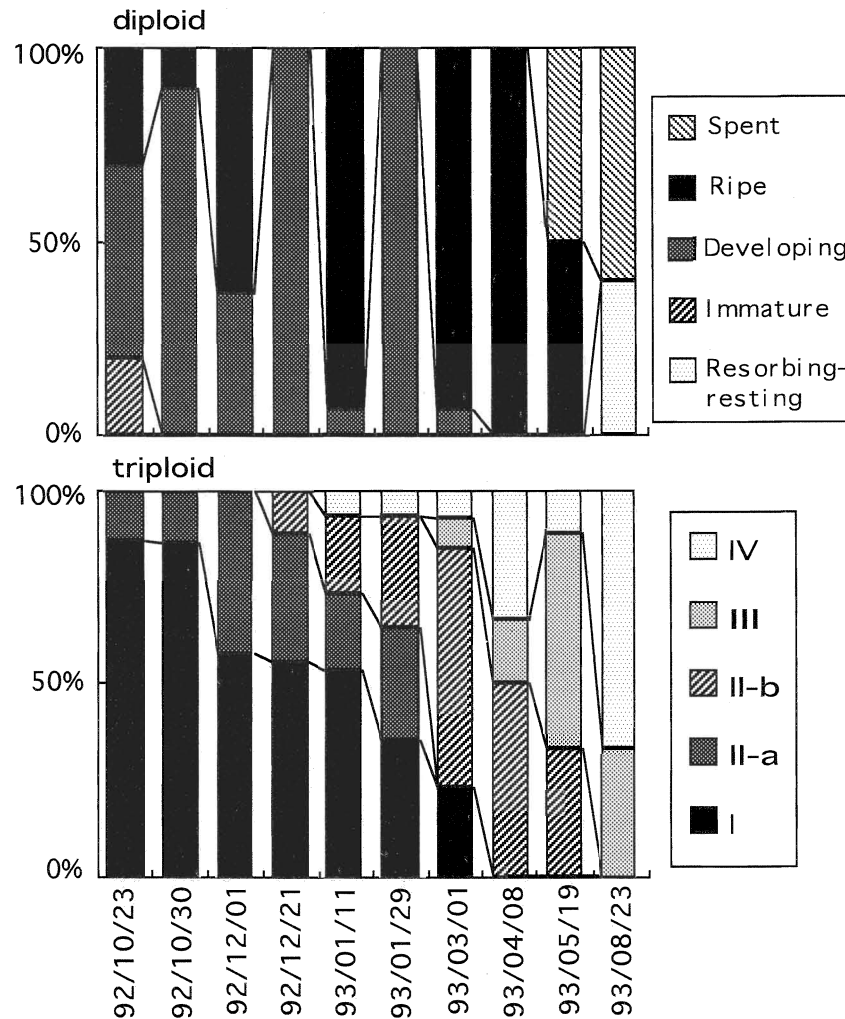


Fig. 2. Frequency distribution of gonadal maturation stages in diploid and triploid mussels. Abscissa: sampling date. Ordinate: percentage of mussels at each stage. The gonadal development of triploid mussels was different from that of diploids, so different stages were used. For details of each stage see Materials and Methods, Results, and Fig. 1.

In triploids it was difficult to observe the transformation process of spermatids to spermatozoa because of their scarcity. In the spermatocyte layer, degenerated germ cells with an irregular cell shape were frequently observed (Figs. 3C and 4A).

Stereological analysis

Comparison of the relative volume fraction of each gonadal cell category indicated that gonadal development in triploids was different from diploids (Fig. 5). In diploid mussels, pre-meiotic cells (spermatogonia and primary spermatocytes) occupied from 1.75 to 33% of the gonad volume fraction, depending on the period of the reproductive cycle (Fig. 5A). In triploid mussels, pre-meiotic cells reached about 40%, which was significantly different from diploids when diploids were maturing. Germ cells after meiosis I (spermatocytes II, spermatids, and spermatozoa) represented from 2 to 61% of the diploid gonad volume fraction, whereas these cell types never exceeded 2% in triploids (Fig. 5B). In triploids, necrotic

spermatocytes and degenerating cells reached about 20% during late December and early January, while this volume fraction was almost non-existent in diploids (Fig. 5C). The somatic cell volume fraction (Sertoli cells and hemocytes) was significantly higher in triploids from 1992/12/01 through 1993/08/23 (Fig. 5D).

Sertoli cells

Sertoli cells could be recognized in triploids by light microscopy (Fig. 3C), whereas in diploids Sertoli cells could only be observed with the aid of electron microscopy (Fig. 4C). The Sertoli cells in mature diploid males rested on the basement membrane of testis acini and extended toward the lumen, coming in contact with spermatogonia, spermatocytes and spermatids. The nucleus was located near the basal part of the cells and rough endoplasmic reticulum was distributed along the cytoplasmic extension. The Golgi apparatus was well developed with fine granules and rough endoplasmic reticulum (Fig. 4D). Sertoli cells in diploids did not show any

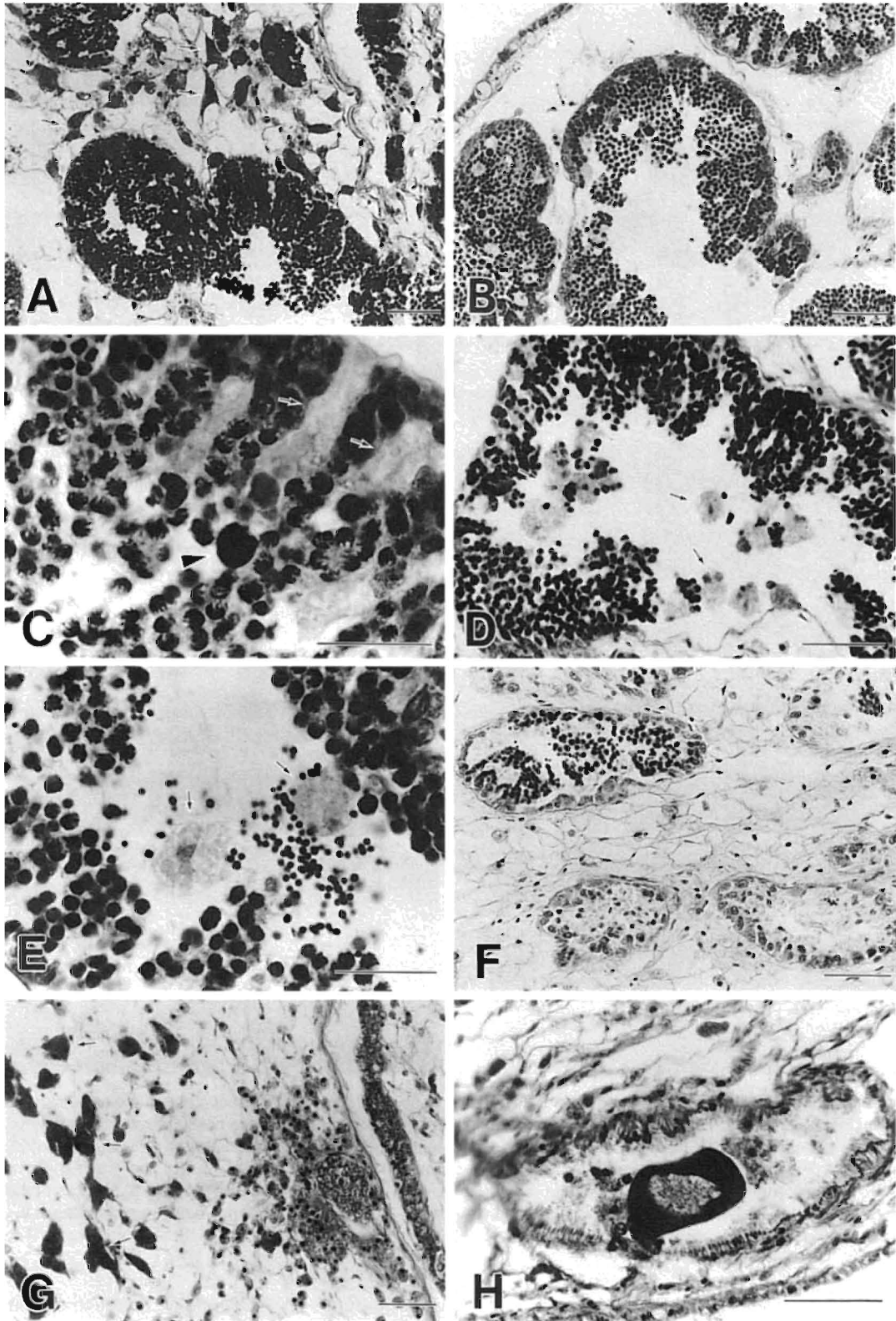


Fig. 3. Micrograph of gonad in triploid mussels. A, October 1992 at stage I. Arrows indicate adipogranular (ADG) cells in connective tissue. B and C, December 1992 at stage II-a. Arrows in C indicate Sertoli cells on the basement membrane in the testicular acini. Arrowhead in C indicates degenerated germ cells. D and E, January 1993 at stage II-b. Arrows indicate hemocytes presumed to phagocytize spermatocytes (D) or spermatozoa (E). F, April 1993 at stage III. G and H, January 1993 at stage IV. Arrows in G indicate reappearing ADG cells in connective tissue. Scale bar indicates 100 μm in A, B, D, F, G, and H, 50 μm in C and E.

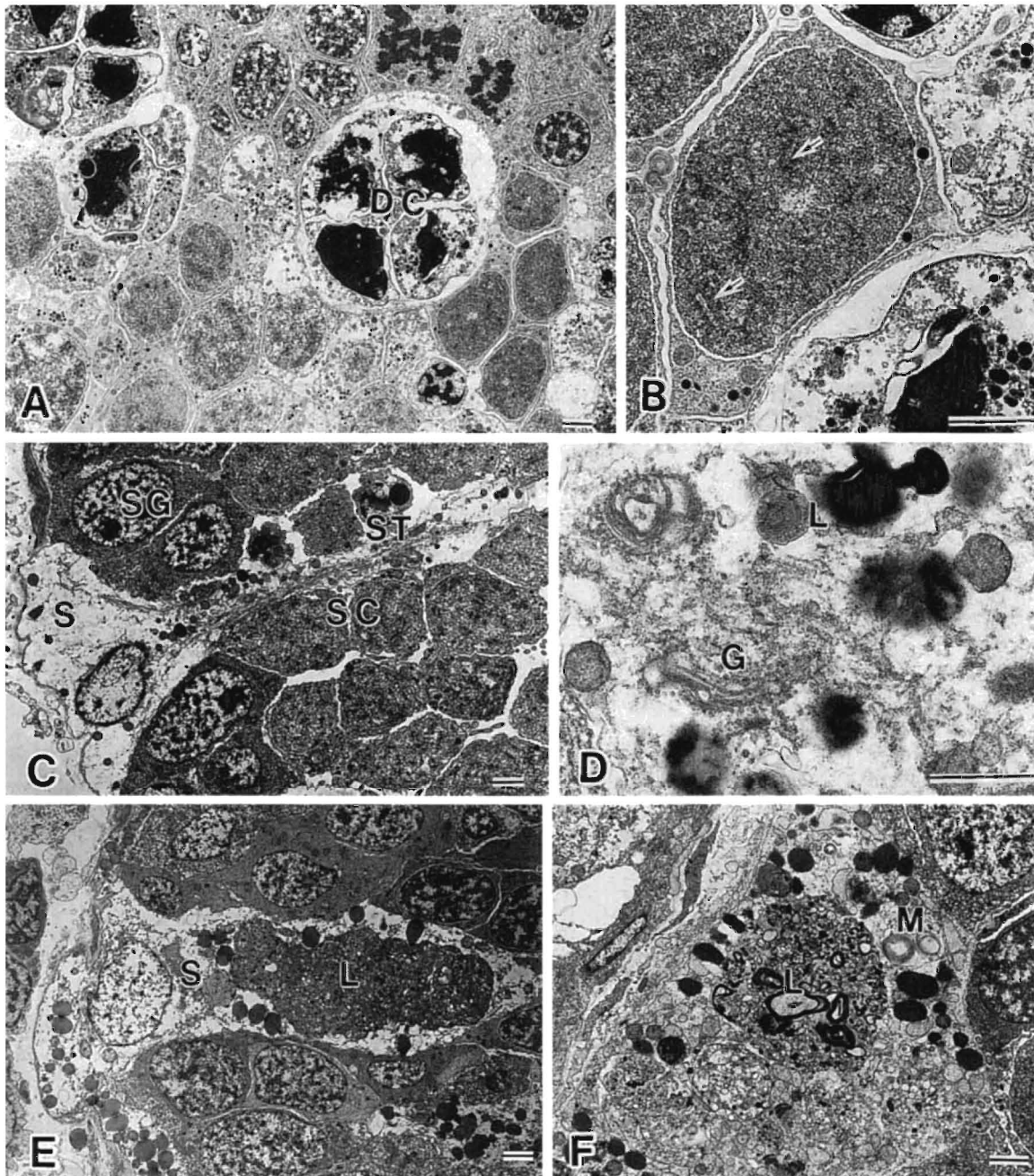


Fig. 4. Transmission electron micrographs of triploid and diploid testis. A, spermatocytes at different stage of meiosis in testis from triploid in January 1993. Degenerated spermatocytes (DC) with pyknotic nucleus. B, the synaptonemal complex (arrows) in spermatocytes. C, testis of diploid mussel in December 1992. Sertoli cell (S) protrudes into testis and it contacts spermatogonia (SG), spermatocytes (SC), and spermatids (ST). D, Sertoli cell cytoplasm in a diploid mussel in December 1992 showing lysosomes (L) and rough endoplasmic reticulum arrangement. The Golgi apparatus (G) and associated fine granules were well developed. E, Sertoli cell (S) in testis of triploid mussel in January 1993. Cytoplasm was filled with large secondary lysosomes (L). F, Cytoplasm of Sertoli cell of triploid mussel in January 1993. Cytoplasm was filled with large lysosomes (L), myelin-like structure (M), and electron dense granules. Scale bar indicates 2 μ m in all figures.

obvious ultrastructural changes from December to April except for a slight increase of lipid-like droplets.

Early in the spawning season, the Sertoli cells in triploids were not noticeably different from those in diploids. In January, Sertoli cells in triploids exhibited an enlarged cytoplasmic area compared to those in diploids (Fig. 4E). Within the cytoplasm of the Sertoli cells were degenerated spermatocytes or

spermatogonia. Additionally, lipid-like droplets and large secondary lysosomes were observed. As shown at high magnification (Fig. 4F), the cytoplasm was filled with large secondary lysosomes, electron dense granules, and myelin-like structures. The rough endoplasmic reticulum displayed an expanded cisternae.

Table 2. Number of spermatozoa per 10000 μm^2

	triploid		diploid	
	Oct. 23	Mar. 1	Oct. 23	Mar. 1
	0	5.1	868.1	1132.4
	0	1.7	1364.9	878.2
	0	19.6	1250.8	1205.6
average	0	8.8	1161.3	1072.1

Note: Each number is the mean value of spermatozoa number observed in three parts of gonad of each individual on the photographs of histological section.

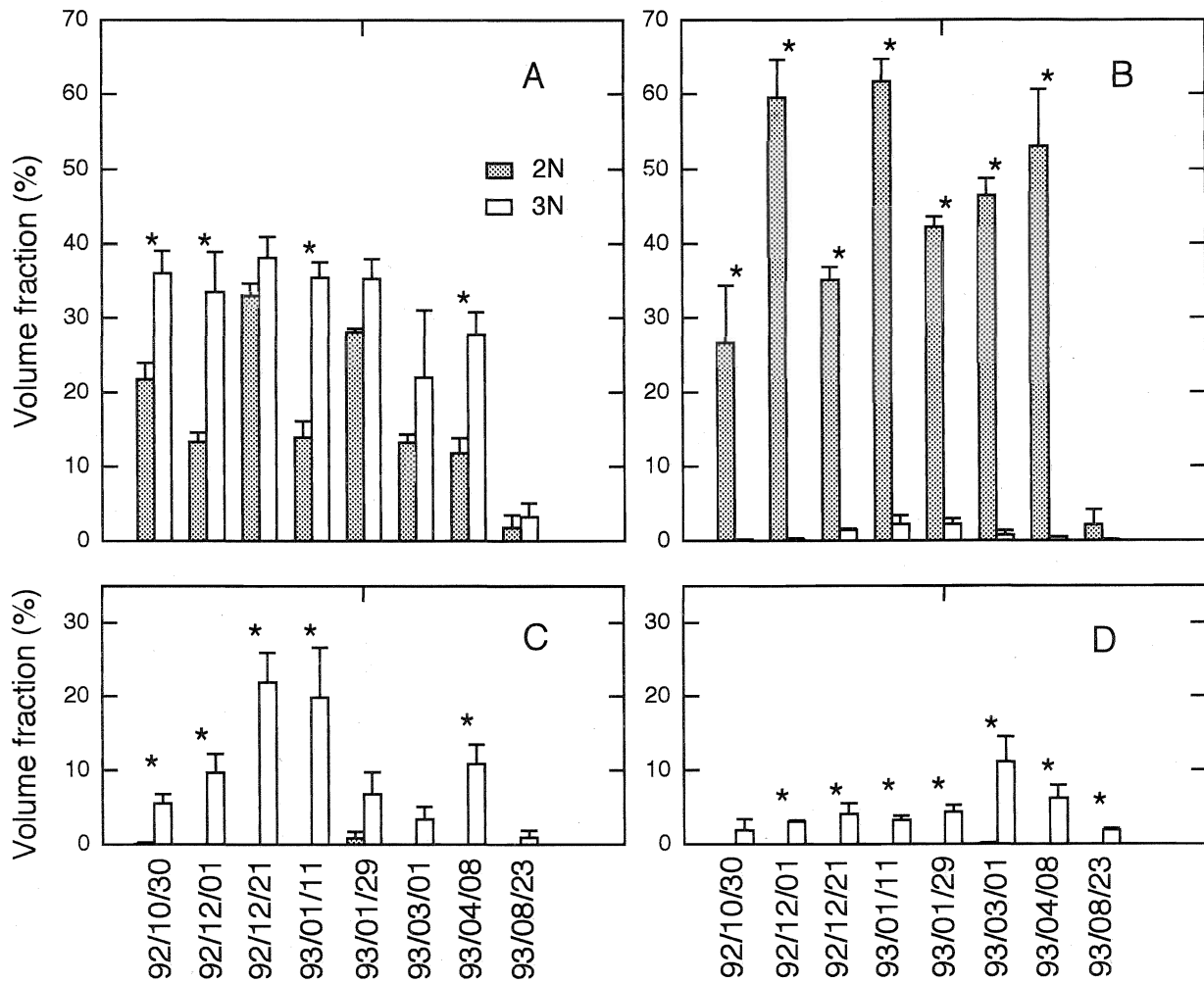


Fig. 5. Comparative evolution of gametogenesis between diploid and triploid mussels: gonadal volume fractions of the different germinal cell categories. A, pre-meiotic germ cells (spermatogonia and primary spermatocytes). B, germ cells after meiosis I (secondary spermatocytes, spermatids and spermatozoa). C, abnormal and degenerating cells. D, somatic cells (Sertoli cells and hemocytes). Values are means \pm S.E.M. * indicates significant differences ($p < 0.05$) between diploid and triploid groups.

DISCUSSION

Suppressed gametogenesis

In the present study, all triploid mussels were male, therefore, only spermatogenesis could be described and compared to normal diploids. In general, spermatogenesis in

triploids was arrested at prophase I of meiosis and eventually the spermatocytes degenerated. This is in agreement with results reported for triploid noble scallop *Chlamys nobilis* (Komaru and Wada, 1988) and soft shell clam *Mya arenaria* (Allen *et al.*, 1986). The sterility of triploid bivalves may be associated with incomplete synapsis of homologous

chromosomes as observed in primary spermatocytes of triploid noble scallop (Komaru, 1989) and triploid carp (Gui *et al.*, 1992).

However, incomplete synapsis of homologous chromosomes may not be the only factor that disrupts the normal control system for meiosis. In eggs from triploid Pacific oyster *Crassostrea gigas* the degree of synapsis varies considerably within and among females (Guo and Allen, 1994a). Although trivalents are the dominant form of synapsed chromosomes, there was no correlation between synapsis and fecundity (Guo and Allen, 1994a). This means that normally synapsed chromosomes are not strictly necessary to obtain mature gametes, at least in female bivalves which release their eggs at the early prophase I stage of meiosis. Consequently, it is difficult to present incomplete synapsis as the main factor for sterility of triploid bivalves (Guo and Allen, 1994a).

In some bivalve species induced triploidy only reduces the amount of mature and functional gametes compared to diploids (Allen and Downing, 1990; Akashige, 1990; Guo and Allen, 1994a,b; Komaru and Wada, 1994). Therefore, genetic or cellular control of gametogenesis varies among bivalve species. In the current study, late in the spawning season, a small proportion (3%) of triploid mussels produced extremely small amounts (2% relative to diploids) of spermatozoa. This implies that the extent of suppression could be different among individuals as well as among species. The relative percentage of cells categorized as secondary spermatocytes, spermatids, and spermatozoa (i.e., having completed first meiosis) was less than 3%. This can be considered an overestimate of the reproductive potential of triploid male *Mytilus galloprovincialis*. The value is close to the 2% relative reproductive potential reported for triploid female *Crassostrea gigas* (Guo and Allen, 1994a). However, gamete production in dwarf surfclams *Mulinia lateralis* was less affected by triploidy, being 80% for males and 59% for females (Guo and Allen, 1994b).

Differences in production of functional gametes by polyploid bivalves may be caused by a number of factors as described for other species. Male tetraploid silkworms are usually sterile, exhibiting an arrest of spermatogenesis at the spermatocyte stage (Kawamura, 1994). Under some experimental conditions, such as high temperature, allatectomy, or starvation, fertile tetraploids were produced (Kawamura, 1994). Fertility was greatly increased when starvation occurred at a specific stage of meiosis (Kawamura *et al.*, 1995). These results indicate that environmental factors which affect the physiology of the organism, and possibly the endocrinological system, can overcome a meiosis block induced by polyploidy.

Gametogenesis in mussels is regulated by a neuroendocrine system (reviewed in Zwaan and Mathieu, 1992) and the production of an insulin-like peptide closely follows the gametogenic cycle (Danton *et al.*, 1996). In the current study, there was equivalent proliferation of spermatogonia to production of spermatocytes between diploids and triploids. Therefore, in bivalve species or

individuals in which triploidy produces virtual sterility, such as the mussel, an endocrinological factor promoting spermatocyte maturation may be greatly disrupted. In species where triploidy does not have such an effect, such as the dwarf surfclam, the endocrinological signal may not be disrupted as much or plays a less significant role in gametogenesis.

Sertoli cells

In diploid mussels, Sertoli cells were too thin to be observed by light microscopy, but their existence on the basement membrane of the gonad was confirmed by transmission electron microscopy. In the triploids, Sertoli cells became so enlarged that they could be observed easily by light microscopy. One of the functions of Sertoli cells is digestion of the residual cytoplasm during spermatogenesis (Pipe, 1987). In triploids, the number of spermatocytes which failed to mature was so great that Sertoli cells exhibited an expanded cytoplasm due to the quantity of spermatocytes and degenerating cells phagocytized. When degeneration of the gonad in triploids began in December hemocytes appeared in the testicular acini. Hemocytes were observed infrequently during the early gametogenetic stage of triploids and diploids. Sertoli cells are supposed to prevent the invasion of hemocytes into testis due to the so-called blood testis barrier (Buckland-Nicks and Chia, 1986). The malfunction of Sertoli cells in maintaining this barrier may have been caused by the Sertoli cells being overwhelmed with phagocytosis of the numerous degenerating spermatocytes, thereby allowing hemocytes to enter the testicular acini and engage in phagocytizing spermatocytes.

Male dominant sex ratio

An unexpected result of induced triploidy in the mussel *M. galloprovincialis* was the production of only male progeny, while the diploid control group had an approximate 1:1 sex ratio. All triploid mussels were examined at the light microscope level and found to contain male features, such as spermatocytes in testicular acini. This biased sex ratio was not caused by the small number of parents, which was eight females and six males, as has been seen with single pair matings of *M. edulis* (Zouros *et al.*, 1994). To reaffirm that the production of only male progeny was not by chance, another trial of induced triploidy was performed one year later with ten females and nine males. The progeny of this cross exhibited the same male dominance in the triploid group and an almost equal proportion of sexes in the diploid group.

The mechanism of sex determination is still unknown in bivalves. Unbiased sex ratios have been observed in triploid noble scallop (Komaru and Wada, 1988), triploid dwarf surfclam (Guo and Allen, 1994b), and triploid Pacific oyster, although hermaphroditism was markedly higher in the Pacific oyster (Allen and Downing, 1990), lending no support for any model. In the American oyster, *Crassostrea virginica*, a sex determination model containing a minimum of 3 genes with additive male and female alleles was proposed (Haley, 1977, 1979). Triploid soft shell clams, *Mya arenaria*, were virtually

all female and the authors proposed that sex determination in *Mya* fit the model of an X:autosome balance mechanism as found for the fruit fly *Drosophila* (Allen *et al.*, 1986). In dwarf surfclams, diploid gynogenesis were all female and triploids had a slight male bias, leading the authors to propose an X:Y sex determination mechanism as found in mammals and other vertebrates (Guo and Allen, 1994b).

The results of the present study indicate a genetic factor in sex determination for *M. galloprovincialis* because ploidy manipulation produced a uniform male sex feature. Environmental influence is not discounted, but is considered less likely since multiple parents were used to produce two cohorts one year apart. It is known in Mytilid mussels that biparental transmission of mitochondrial DNA (mtDNA) to offspring occurs (Fisher and Skibinski, 1990; Zouros *et al.*, 1992). Females usually contain only one haplotype, which is inherited from their mother, whereas all males in a homospecific cross are heteroplasmic, containing mtDNA from both the mother and father (Fisher and Skibinski, 1990; Zouros *et al.*, 1992). Fisher and Skibinski (1990) reported that gonadal tissue of males contained only the paternally derived haplotype. Zouros *et al.* (1994) conclusively showed that mtDNA inheritance is associated with a strong sex ratio bias and is not a mechanism of sex determination.

The all male triploid progeny of the present study indicates that a Z:W or Z:O model for sex determination, as found in avian species, could be contemplated. In this model the female is heterogametic and sex is determined by the maternal gamete. Triploid chickens are all males and gametogenesis is suppressed (Abdel-Hameed and Shoffner, 1971). Their left gonad displayed an ovarian histology at hatching that degenerated 6 months later and was subsequently replaced by a testis (Solari, 1994). This gonadal sex reversal may have occurred by a minor ZZ line amplification or a mitotic loss of the W (Solari, 1994). In triploid plaice *Pleuronectes platessa* a high percentage (95%) of males was observed (Lincoln, 1981), yet the occurrence of both males and females in gynogenetic diploids (Purdom and Lincoln, 1973) implies that in this species the female is heterogametic and a Z:W (or Z:O) sex determining mechanism occurs. Sex determination in the newt, *Triturus viridescens*, follows a Z:W model (Griffiths, 1941). Triploids displayed a female sex ratio bias and it was proposed that post-reductive meiosis was the mechanism for this bias (Griffiths, 1941). In mussels, there is evidence that the sex ratio is maternally influenced (E. Zouros, personal communication). The male-dominant sex ratio found in the triploid mussels of the present study may have resulted from post-reductive meiosis as found in the newt, or by some additional factor(s), such as an altered balance between sex chromosomes and autosomes, or a combination of mechanisms.

ACKNOWLEDGMENTS

Support for M. Kiyomoto, E. Danton, and J. Scarpa was provided by the Science and Technology Agency of Japan and for J. Scarpa

by National Science Foundation (USA) under grant No. 91-01762. We are grateful to Dr. M. Mathieu of Universite de Caen for his support of this research and to Ms. T. Okumura for her assistance during sampling.

REFERENCES

- Abdel-Hameed F, Shoffner RN (1971) Intersexes and sex determination in chickens. *Science* 172:962–963
- Akashige S (1990) Growth and reproduction of triploid Japanese oyster in Hiroshima Bay. In "Advances in Invertebrate Reproduction Vol 5" Ed by M Hoshi, O Yamashita, Elsevier, Amsterdam, pp 461–468
- Allen Jr SK, Downing SL (1990) Performance of triploid Pacific oysters, *Crassostrea gigas*: gametogenesis. *Can J Fish Aquat Sci* 47: 1213–1222
- Allen Jr SK, Hidu H, Stanley JG (1986) Abnormal gametogenesis and sex ratio in triploid soft-shell clams (*Mya arenaria*). *Biol Bull* 170: 198–210
- Beaumont AR, Fairbrother JE (1991) Ploidy manipulation in molluscan shellfish: a review. *J Shellfish Res* 10: 1–18
- Buckland-Nicks J, Chia FS (1986) Fine structure of Sertoli cells in three marine snails with a discussion on the functional morphology of Sertoli cells in general. *Cell Tissue Res* 245: 305–313
- Danton E, Kiyomoto M, Komaru A, Wada KT, Awaji M, Mathieu M (1996) Comparative analysis of storage tissue and insulin-like neurosecretion in diploid and triploid mussels *Mytilus galloprovincialis* LMK in relation to their gametogenesis cycle. *Inver Reprod Dev* 29, in press
- Fisher C, Skibinski DOF (1990) Sex-biased mitochondrial DNA heteroplasmy in the marine mussel *Mytilus*. *Proc Royal Soc Lond (B)* 242:149–156
- Griffiths RB (1941) Triploidy (and haploidy) in the newt *Triturus viridescens*, induced by refrigeration of fertilized eggs. *Genetics* 26: 69–88
- Gui JF, Jia J, Liang SC, Jiang YG (1992) Meiotic chromosome behavior in male triploid transparent colored crucian carp, *Carassius auratus* L. *J Fish Biol* 41: 317–326
- Guo X, Allen Jr SK (1994a) Reproductive potential and genetics of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). *Biol Bull* 187: 309–318
- Guo X, Allen Jr SK (1994b) Sex determination and polyploid gigantism in the dwarf surfclam (*Mulinia lateralis* Say). *Genetics* 138: 1199–1206
- Jong-Brink M, Schot LPC, Shoenmakers HJN, Bergamin-Sassen MJM (1981) A biochemical and quantitative electron microscope study on steroidogenesis in ovotestis and digestive gland of the pulmonate snail *Lymnaea stagnalis*. *Gen Comp Endocrinol* 45: 30–38
- Haley L (1977) Sex determination in the American oyster. *J Heredity* 68: 114–116
- Haley L (1979) Genetics of sex determination in the American oyster. *Proc Natl Shellfisheries Association* 69: 54–57
- Kawamura N (1994) Male meiosis in polyploid silkworms, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Int J Insect Morphol Embryol* 4: 311–317
- Kawamura N, Sahara K, Iisuka T (1995) Increase of fertility in tetraploid male silkworm by starvation. *Hereditas* 122: 119–123
- King PA, McGrath D, Gosling EM (1989) Reproduction and settlement of *Mytilus edulis* on an exposed rocky shore in Galway Bay, west coast of Ireland. *J Mar Biol Ass UK* 69: 355–365
- Komaru A (1989) Incomplete synapsis in spermatocytes of induced triploid scallops, *Chlamys nobilis* (Bivalvia: Pterimorphia). *Chromosome Information Service* 47: 7–9
- Komaru A, Konishi K, Wada KT (1994) Ultrastructure of spermatozoa from induced triploid Pacific oyster, *Crassostrea gigas*.

- Aquaculture 123: 217–222
- Komaru A, Uchimura Y, Ieyama H, Wada KT (1988) Detection of induced triploid scallop, *Chlamys nobilis*, by DNA microfluorometry with DAPI staining. Aquaculture 69: 201–209
- Komaru A, Wada KT (1988) Gametogenesis and growth of induced triploid scallops *Chlamys nobilis*. Nippon Suisan Gakkaishi 55: 477–452
- Komaru A, Wada KT (1990) Gametogenesis of triploid Japanese pearl oyster, *Pinctada fucata martensii*. In "Advances in Invertebrate Reproduction Vol 5" Ed by M Hoshi, O Yamashita, Elsevier, Amsterdam, pp 469–474
- Komaru A, Wada KT (1994) Meiotic maturation and progeny of oocytes from triploid Japanese pearl oysters (*Pinctada fucata martensii*) fertilized with spermatozoa from diploids. Aquaculture 120: 61–70
- Lincoln RF (1981) Sexual maturation in female triploid plaice, *Pleuronectes platessa*, and plaice × flounder, *Platichys flesus*, hybrids. J Fish Biol 19: 499–507
- Lowe DM, Moore MN, Bayne BL (1982) Aspects of gametogenesis in the marine mussel *Mytilus edulis* L. J Mar Biol Ass UK 62:133–145
- Pechevik JA, Eyster LS, Widdows J, Bayne BL (1990) The influence of food concentration and temperature on growth and morphological differentiation of blue mussel *Mytilus edulis* L. larvae. J Exp Mar Biol Ecol 136: 47–64
- Pipe RK (1987) Ultrastructural and cytochemical study on interactions between nutrient storage cells and gametogenesis in the mussel *Mytilus edulis*. Marine Biol 96: 519–528
- Purdom CE, Lincoln RF (1973) Chromosome manipulation in fish. In "Genetics and Mutagenesis of Fish" Ed by Schroder JH, Springer Verlag, Berlin, pp 83–89
- Scarpa J, Toro JE, Wada KT (1994) Direct comparison of six methods to induce triploidy in bivalves. Aquaculture 119: 119–133
- Solari AJ (1994) Sex Chromosomes and Sex Determination in Vertebrates. CRC Press, Boca Raton, pp 62–71
- Weibel ER, Kistler GS, Scherle WF (1966) Practical stereological methods for morphometric cytology. J Cell Biol 30: 23–38
- Wilson JH, Seed R (1974) Reproduction in *Mytilus edulis* L. (Mollusca: Bivalvia) in Carlingford Lough, Northern Ireland. Irish Fisheries Investigations Series B No 15: 3–30
- Zouros E, Freeman KR, Ball AO, Pogson GH (1992) Direct evidence for extensive paternal mitochondrial DNA inheritance in the blue mussel *Mytilus*. Nature 359: 412–414
- Zouros E, Ball AO, Saavedra C, Freeman KR (1994) An unusual type of mitochondrial DNA inheritance in the blue mussel *Mytilus*. Proc Natl Acad Sci USA 91: 7463–7467
- Zwaan AD, Mathieu M (1992) Metabolism and gametogenesis: Neuroendocrine regulation. In "The Mussel *Mytilus*: Ecology, Physiology, Genetics and Culture" Ed by E Gosling, Elsevier, Amsterdam, pp 272–292

(Received January 20, 1996 / Accepted March 15, 1996)