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Sex Determination and Polyploid Gigantism in the Dwarf Surfclam (*Mulinia lateralis* Say)

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Manuscript received February 1, 1994
Accepted for publication August 20, 1994

ABSTRACT

Mulinia lateralis, the dwarf surfclam, is a suitable model for bivalve genetics because it is hardy and has a short generation time. In this study, gynogenetic and triploid *M. lateralis* were successfully induced. For gynogenesis, eggs were fertilized with sperm irradiated with ultraviolet light and subsequently treated with cytochalasin B to block the release of the second polar body (PB2). Triploidy was induced by blocking PB2 in normally fertilized eggs. The survival of gynogenetic diploids was very low, only 0.7% to 8 days post-fertilization (PF), compared with 15.2% in the triploid groups and 27.5% in the normal diploid control. Larvae in all groups metamorphosed at 8–10 days PF, and there was no significant post-larval mortality. At sexual maturation (2–3 months PF), all gynogenetic diploids were female, and there was no significant difference ($P > 0.05$) in sex ratio between diploids and triploids. These results suggested that the dwarf surfclam may have an *XX*-female, *XY*-male sex determination with *Y*-domination. Compared with diploids, triploids had a relative fecundity of 59% for females and 80% for males. Eggs produced by triploid females were 53% larger ($P < 0.001$) in volume than those from diploid females. In both length and weight measurements at three months PF, the gynogenetic diploids were not significantly ($P > 0.33$) different from normal diploid females, suggesting that inbreeding depression was minimal in meiosis II gynogens. Triploid clams were significantly larger ($P < 0.001$) than normal diploids. We hypothesize that the increased body-size in triploids was caused by a polyploid gigantism due to the increased cell volume and a lack of cell-number compensation.

OVER the last decade, chromosome set manipulation in mollusks has received much attention. Most of the research has focused on the development of techniques for producing triploids and gynogens, primarily intended for potential applications in aquaculture. Triploids have been successful in the culture of species such as the Pacific oyster *Crassostrea gigas*, for which sexual maturation of diploids is a problem for marketing (ALLEN *et al.* 1989). Gynogens have potential for the rapid production of inbred lines (PURDOM 1983).

Chromosome set manipulation is also an important tool for genetic analysis. The production of polyploids has been useful in studies on effects of changes in chromosome number on development (FANKHAUSER 1945). There have been attempts to evaluate the evolutionary significance of polyploidy in animals, but they are limited by our poor understanding of the biology and reproductive genetics of polyploid animals (SCHULTZ 1980; BOGART 1980; GUO and ALLEN 1994a). The production of gynogens can be used for gene mapping and to study genetic sex determination which is still largely unknown in mollusks (HALEY 1977; 1979; ALLEN *et al.* 1986).

In mollusks, triploidy has been induced in over a dozen of species (see review by BEAUMONT and FAIRBROTHER 1991). Successful gynogenesis were also reported in the Pacific abalone (FUJINO *et al.* 1990) and the Pacific oyster (GUO *et al.* 1993). Recently, viable tetraploids were also reported in the blue mussel (SCARPA *et al.* 1993) and the Pacific oyster (GUO and ALLEN 1994b). Those developments suggest that techniques of chromosome set manipulation have reached a level of maturity in mollusks. However, the performance of triploid, tetraploid and gynogenetic diploid mollusks has not been well documented. The growth of triploids is relatively well studied and has been reported in several species (BEAUMONT and FAIRBROTHER 1991). Studies on the sterility of triploids have been limited primarily to histological examination of gonads. Fecundity and reproductive potential are estimated in only one species, the Pacific oyster (GUO and ALLEN 1994a). Observations on the performance of tetraploid and gynogenetic mollusks are very preliminary (FUJINO *et al.* 1990; GUO and GAFFNEY 1993; SCARPA *et al.* 1993; GUO and ALLEN 1994b). A major difficulty in evaluating the performance of polyploid and gynogenetic mollusks is their long generation time, usually 1–2 years. To overcome this constraint, we used the dwarf surfclam (*Mulinia lateralis* Say) as a model species to study effects of various chromosome set manipulations. *M. lateralis* has a short generation time of about 3 months and is easily cultured in the laboratory (CALABRESE 1969). In this study, *M. lateralis* was used to document performance of gynogens and triploids.

MATERIALS AND METHODS

M. lateralis parents used in this study were the F_1s from a random mating of animals obtained from the state of Virginia.

Gametes were obtained by dissecting gonads, and the somatic tissue was saved for allozyme analysis. Eggs were passed through a 85-μm Nytex screen to remove large tissue debris, and rinsed on a 25-μm screen. Sperm suspensions were passed through a 15-μm screen to remove large debris. Seawater used for rearing larvae was filtered to 2 μm, and the salinity was about 25 ppt. Fertilization and treatments were conducted at 24–26°.

Gynogenesis was induced by fertilizing eggs with sperm irradiated by ultraviolet (UV) light. Two 8-watt shortwave UV tubes (G8T5, VWR) were used in the irradiation chamber. To determine the appropriate UV dosage, sperm were exposed to UV light for 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0 min at an intensity of 1300–1500 $\mu W/cm^2$. The UV intensity was measured by a Black-Ray UV meter (J225, UVP, Inc.) and adjusted by varying the distance between sperm and UV light tube. For the dosage experiment, eggs from four females were pooled and then divided equally into nine groups, and sperm from six males were combined. The sperm density was adjusted to about 100 million/ml at a standard depth of 1 mm (Guo *et al.* 1993). The success of gynogenesis was judged by fertilization success and development of haploids. Fertilization success was determined at 90–120 min post-fertilization (PF) as the proportion of eggs undergoing polar body release or cleavage. The induction of haploid development was determined by chromosome counts of 20 2–4-cell embryos per group (Guo and Allen 1994a). The optimum UV dosage was used to produce gynogenetic diploids in subsequent experiments.

Gynogenetic diploids were induced by blocking polar body II (PB2) with cytochalasin B (CB) in eggs fertilized with UV-irradiated sperm (Thorgaard 1983; Guo *et al.* 1993). Triploids were induced by blocking PB2 with CB in eggs fertilized with untreated sperm (Allen *et al.* 1989). To block the release of PB2, fertilized or activated eggs were treated with 0.5 mg/liter CB dissolved in 0.5 ml dimethyl sulfate (DMSO), for 15 min beginning at 30 min PF. Four experimental groups were produced with each pair of parents (replicate): 1n, a haploid group in which eggs were fertilized with UV-irradiated sperm and allowed to develop without CB treatment; 2n, a normal fertilization used as a diploid control; 2ng, a gynogenetic diploid group; and 3n, a triploid group. The four groups were replicated five times using different pairs of parents.

In each replicate, chromosome number of 100 embryos (2–4 cells) in the haploid groups was determined to verify the inactivation of sperm chromosomes. Embryos were cultured in 15-liter buckets at a density of 50–67 embryo/ml; approximately equal density was maintained in all four groups. Ploidy of 24-hr-old larvae was analyzed by flow cytometry and survival of fertilized eggs to D stage (24-hr PF), day 3 and day 8 was determined for all groups. For counting, larvae were collected on a Nytex screen of proper size and rinsed to a known volume of seawater (usually 1–10 liters). Three subsamples of 1 ml were placed in counting chambers and counted under microscope. The average of the three subsamples was used to determine the total number of larvae in that group. After metamorphosis (8–10 days PF), clams were transferred to recirculating systems with sand substrate where all groups within each replicate shared the same feeding reservoir, and approximately the same amount (liters) of algae were delivered to each reservoir.

At 3 months PF, clams in all groups were sampled. The ploidy of sampled clams were determined by flow cytometry (Allen 1983; Guo *et al.* 1993). Length and whole body weight (wet) were measured. Sex was determined by microscopic examination of gonads. Fecundity of a subsample of clams was also determined by dissecting gonads and enumerating gametes obtained. For females, eggs were suspended in 750 ml of seawater and counted in two subsamples of 0.5 ml. For males, sperm were suspended in 1 liter of seawater, and four to five subsamples were counted using a hemacytometer.

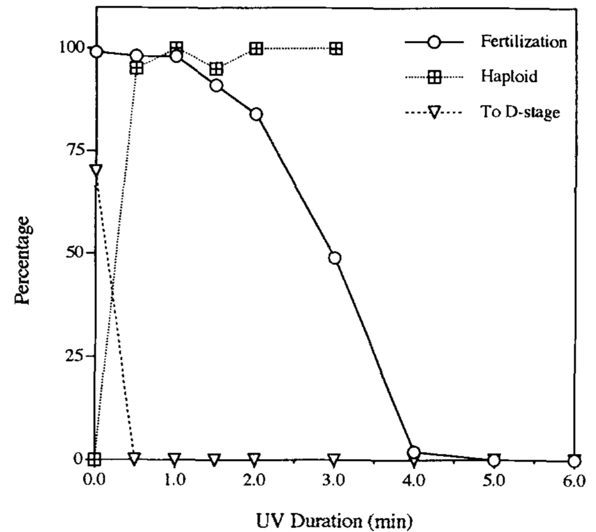

FIGURE 1.—Fertilization, survival and induction of haploids with sperm of *M. lateralis* exposed to different durations of UV light (1400 $\mu W/cm^2$).

RESULTS

UV dosage: Fertilization success decreased gradually with increased UV dosage (Figure 1), decreasing to 49% when sperm were irradiated for 3 min. With 4 min of UV irradiation, only 2% of the eggs developed, and exposures of 5 min or longer completely destroyed the sperm's ability to activate eggs.

Chromosome numbers in the resulting embryos indicated that all UV dosages tested in this study were effective in inactivating sperm chromosomes (Figure 1). Even when sperm were irradiated for as short as 30 sec, no diploid development occurred. The dwarf surfclam has a diploid chromosome number of 38 (1n = 19) (Wada *et al.* 1990). Out of 20 embryos analyzed from the 30-sec irradiation, 19 had exactly 19 chromosomes, and one had 20 chromosomes. Among all embryos (100) analyzed from five treated groups, 97 had 19 chromosomes, one had 18 chromosomes and two had 20 chromosomes. Examples of haploid and diploid metaphases are presented in Figure 2. The UV-dosage experiment was not repeated because of the consistency of the initial experiment. A dose of 1.5-min UV irradiation (1400 $\mu W/cm^2$) to sperm (10^8/ml, 1-mm thick) was considered optimum for inactivation and used for subsequent experiments. In the untreated control, no haploids were found, and 95% of the embryos had exactly 38 chromosomes. One embryo in the control group had 39 chromosomes.

As expected from the chromosome data, survival to D stage dropped sharply to zero at a UV duration as low as 0.5 min (Figure 1), suggesting haploids are incapable of reaching D stage. With high dosages used in this study,

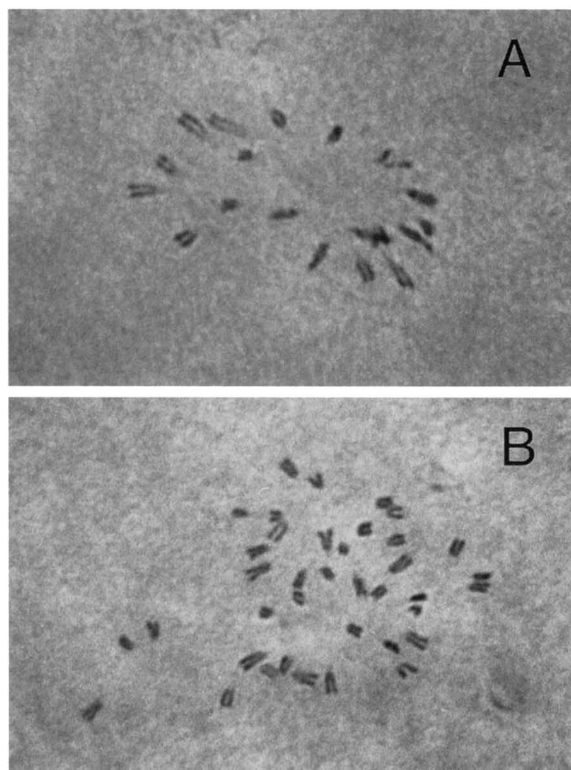

FIGURE 2.—Metaphases from (A) haploid 2–4 cell embryos of *Mulinia lateralis* produced by UV-irradiated sperm (1n = 19), and from (B) gynogenetic diploid produced from retention of the second polar body after CB treatment (2n = 38).

we saw no evidence for spontaneous increase in survival known as the Hertwig effect.

Induction and survival of gynogens and triploids: The UV treatment used in the production of gynogenetic diploids effectively inactivated sperm chromosomes. Chromosome numbers were determined for embryos in three of the five haploid groups (replicates 1, 3 and 4), 100 per group. The 300 embryos analyzed consisted of 281 (93.7%) haploids, 18 (6.0%) aneuploids and 1 (0.3%) triploid. All aneuploids had low chromosome numbers close to haploidy (1n = 19): 5 (1.7%) with 18 chromosomes, 10 (3.3%) with 20 and 2 (0.7%) with 21. One aneuploid (0.3%) was mosaic with 18 and 20 chromosomes.

The ability of the sperm to fertilize eggs was unaffected by UV treatment, and fertilization in all groups was high, ranging from 91 to 100% (Table 1). Survival of fertilized eggs was variable among 1n, 2n, 2ng and 3n groups. In the normal diploid control (2n), the average survival to D stage was 62.2%. Haploid groups (1n) had no survival to D stage in all five replicates. On the other hand, blocking PB2 in the gynogenetically activated eggs (2ng) allowed 3.8% of them to survive to D stage, ranging from 0.5 to 7%. In triploid groups (3n), an average of 20.1% of the fertilized eggs developed into D stage (Table 1). The same pattern of survival persisted to day 3 and day 8 PF. At day 8 PF, cumulative survival was, on the average, 27.5% for the diploid control, 0.7% for the gynogenetic groups, and 15.2% for the triploid groups.

TABLE 1

Number of eggs used, percent fertilization and cumulative survival of fertilized zygotes to D stage, day 3 and day 8 in experimental groups by replicates

Group[a]	Eggs (10^3)	Fertilization	D stage	Day 3	Day 8
1n 1	170	97	0		
2	69	100	0		
3	98	95	0		
4	30	95	0		
5	63	91	0		
Mean			0		
2n 1	170	100	70.0	65.2	13.0
2	69	100	68.1	68.1	
3	94	98	66.9	63.9	30.9
4	30	100	40.4	40.4	38.6
5	63	98	65.5	58.6	
Mean			62.2	59.2	27.5
2ng 1	567	91	1.9	0.3	0.3
2	411	100	5.0	0.8	
3	983	96	7.0	2.0	1.5
4	301	98	4.5	1.0	0.4
5	634	93	0.5	0.04	
Mean			3.8	0.8	0.7
3n 1	283	100	3.6	2.0	2.0
2	137	100	11.4	11.4	
3	295	96	44.3	40.0	31.1
4	60	98	29.3	28.5	12.4
5	127	98	11.7	8.8	
Mean			20.1	18.1	15.2

[a] 1n = gynogenetic haploid; 2n = diploid control; 2ng = gynogenetic diploid; and 3n = triploid.

Replicates 2 and 5 were discontinued at day 3 due to low survival, lack of chromosome counts of haploids, and space limitations. Larvae in the remaining three replicates (2n, 2ng and 3n groups per replicate) metamorphosed at day 8–10 PF and were reared to sexual maturation. After metamorphosis, young clams were transferred to a recirculating system for rearing. There was virtually no post-larval mortality.

At day 30 PF, the ploidy of 25 clams were determined in each group by flow cytometry. Clams from the diploid control and gynogenetic groups were all diploid. In triploid groups, diploids and triploids were the only types of ploidy identified. The percentages of triploids were 88, 33 and 100% for the three replicates (Table 2). Approximately the same percentages of triploids were observed at 3 months PF in all three replicates.

Sex ratio, fecundity and body size: Gametes were observed in the majority of clams in all groups at three months PF. The percentage of immature clams was approximately the same in all experimental groups (Table 3). Diploid clams were 40% female, 50% male and 10% immature. If we could assume the immature clams were females, the diploids would have an exact 1:1 sex ratio. In the gynogenetic diploid groups, however, not a single male was found among 225 clams examined, and all clams whose sex could be determined were females. Immature clams accounted for 16% of gynogens. Although

TABLE 2

Percent triploids induced in three replicates determined by flow cytometry at one and three months post-fertilization (PF)

	1 Month PF		3 Months PF	
Replicate	*n*	%	*n*	%
1	25	88	75	92
3	15	33	75	21
4	25	100	75	100

TABLE 3

Sex-ratio of normal diploid (2n), gynogenetic diploid (2ng), and triploid (3n) *M. lateralis* at three month of age

Group	Female (%)	Male (%)	No gametes (%)	Total
2n	89 (40)	113 (50)	23 (10)	225
2ng	188 (84)	0 (0.0)	37 (16)	225
3n	46 (29)	93 (58)	21 (13)	160

the difference in sex ratio between diploids and triploids was not statistically significant ($P = 0.09$), triploids did show a significant ($P < 0.05$) deviation from the 1:1 sex ratio favoring more males, even assuming all immature clams were females. No hermaphrodites were observed in any group in this study.

On average, normal diploid *M. lateralis* produced 3×10^5 eggs or 2.5×10^9 sperm per individual (Table 4). Comparatively, gynogenetic diploids produced fewer eggs (2.4×10^5), which corresponds to a relative fecundity of 79%. The difference in fecundity between gynogenetic and normal diploids was not statistically significant however ($P = 0.36$). On the other hand, triploid females produced significantly ($P = 0.03$) fewer eggs (1.7×10^5), which correspond to a relative fecundity of 59%. Triploid males produced fewer sperm with a relative fecundity of 80% (Table 4), but the differences between triploid and diploid males was not statistically significant ($P = 0.10$). When fecundity was analyzed per unit body mass, however, both triploid males and females had significantly ($P = 0.000$ and $P = 0.001$) reduced fecundity than normal diploids.

Diameters of ten eggs were measured for 36 normal diploid, 17 gynogenetic diploid, and 24 triploid females. Eggs from normal diploids averaged 49.1 ± 3.1 (SD) μm, and eggs from gynogenetic females were approximately the same size, 48.6 ± 3.1 μm. Eggs from triploids which measured 56.6 ± 3.5 μm in diameter were significantly ($P < 0.000$) larger than those of normal diploids. Compared with normal eggs, eggs from triploids were 15.3% larger in diameter, an estimated 53% increase in volume.

At 3 months PF, length and weight measurements of 75 clams were taken for each group. Clams from triploid groups were individually confirmed by flow cytometry. Diploids from triploid groups (6, 59 and 0 for three replicates, respectively) were excluded because of the possibility that they might be genetic heterogeneous and different from normal diploid controls (due to gynogenesis, for example). Effects of gynogenesis and triploidization were tested in two separate analyses (Table 5). For analysis on gynogenesis, only gynogenetic and normal diploid females were used. In both length and weight measurements, analysis of variance (ANOVA) showed that gynogenesis had no significant effects ($P > 0.80$). Effects due to replicate (or family) were significant for length ($P = 0.001$), but not for weight ($P = 0.184$) (Table 5). Variation among replicates might be caused by genetic differences among females or random environmental factors during replication. The interaction between gynogenesis and replicate was also significant for both measurements ($P < 0.01$). In fact, when three replicates were examined individually with a two sample *t*-test (at 95% confidence level), gynogens were significant smaller than normal diploids in the first replicate (11.9 mm and 315 mg *vs.* 13.1 mm and 410 mg), significantly larger than normal diploids in the second replicate (14.1 mm and 434 mg *vs.* 13.2 mm and 359 mg), and not significantly different from normal diploids in the third replicate (13.0 mm and 367 mg *vs.* 13.0 mm and 371 mg). Apparently, this interaction was due to variation in gynogens, not normal diploid females. Overall, gynogenetic females measured 12.9 mm and 367 mg, which were not significantly different from the 13.1 mm and 372 mg of normal diploid females when tested by a two sample *t*-test ($P = 0.332$ for length, $P = 0.794$ for weight) (Table 6). Interestingly, gynogens (including immatures) were significantly ($P < 0.01$) smaller than normal diploids of both sexes (including immatures), suggesting males were larger than females in normal diploids.

For analysis on triploidization, all individuals from diploid and triploid groups were used (including immature clams). In both length and weight measurements, the ANOVA showed that effects due to ploidy (diploid or triploid) were significant ($P = 0.012$ for length; $P = 0.005$ for weight). Effects due to replicate were significant for weight ($P = 0.01$), but not for length ($P = 0.085$) (Table 5). There was no significant interaction

TABLE 4

Fecundity of normal diploid (2n), gynogenetic diploid (2ng) and triploid (3n) *M. lateralis* at three month of age

	Group	*n*	Mean[a]	CV%	Significance[b]
Female	2n	25	297.4	55	b
	2ng	27	235.4	82	ab
	3n	24	174.3	70	a
Male	2n	25	2.487	51	
	3n	10	2.001	23	

[a] Means for females = $\times 10^3$, and means for males = $\times 10^9$.
[b] Letters designate significant differences in means among females, as indicated by the Tukey HSD multiple comparison test at a confidence level of 95%. The difference between male 2n and 3n was not significant ($P > 0.05$).

TABLE 5

Analysis of variance for length and weight of three families of gynogenetic (2ng), diploid (2n) and triploid (3n) *M. lateralis* groups

Source	d.f.	Length (mm)			Weight (mg)		
		MS	*F* ratio	*P*	MS	*F* ratio	*P*
Gynogenesis:							
Gyno (*G*, fixed)	1	1.0	0.1	0.825	62	0.0	0.980
Replicate (*R*, random)	2	25.9	7.2	0.001	32,708	1.7	0.184
$G \times R$	2	19.7	5.5	0.004	127,238	6.6	0.002
Error	271	3.6			19,215		
Triploidization:							
Ploidy (*P*, fixed)	1	524.6	70.9	0.012	4,729,588	307.1	0.005
Replicate (*R*, random)	2	15.5	2.5	0.085	234,837	4.6	0.010
$P \times R$	2	7.4	1.2	0.309	15,400	0.3	0.738
Error	379	6.3			50,656		

Effects of gynogenesis and triploidization were tested in two separate analyses. For gynogenesis, only females from gynogenetic and normal diploid groups were used. For triploidization, all individuals from triploid and normal diploid groups were used.

TABLE 6

Length and weight measurements of gynogenetic (2ng), normal diploid (2n) and triploid (3n) *M. lateralis* Say at 3 months of age

Group	*n*	Mean	CV (%)	*P* value[a]
Length (mm)				
2n female	89	13.1	14.1	
2ng female	188	12.9	16.2	0.332
2n all[b]	225	13.3	15.9	
3n all	160	16.2	18.5	0.000
Weight (mg)				
2n female	89	372	37.6	
2ng female	188	367	39.6	0.794
2n all	225	390	42.6	
3n all	160	672	43.6	0.000

[a] The *P* value is from a two-sample *t*-test.
[b] For 2n and 3n, all female, male and immature clams were included.

between ploidy and replicate in both length ($P = 0.31$) and weight ($P = 0.74$) measurements, suggesting triploids were always different from diploids regardless of replicates (or families). Sex was not included as a factor in the ANOVA because of the presence of immature clams and their differential distribution among three replicates. The immature clams were small individuals that had not reached sexual maturation, and their exclusion would have created bias in the ANOVA. Although sex could not be included in the ANOVA, it was apparent that the two sexes were different in body size. For all normal diploids whose sex was identified, males averaged at 14.0 mm and 439 mg, females averaged 13.1 mm and 371 mg, and the difference was significant ($P < 0.01$) when tested by a two sample *t*-test. In triploids, males were also larger than females, and the difference was significant in weight ($P = 0.02$), but not in length ($P = 0.10$).

Triploids were significantly ($P < 0.001$) larger than diploids in either sexes. Overall (including both sexes and immature ones), triploids averaged at 16.2 mm and 672 mg, which were 22 and 72% larger than normal diploids (13.3 mm and 390 mg) in length and weight, respectively (Table 6). The difference was highly significant ($P = 0.000$) as indicated by a two-sample *t*-test. The size difference between triploids and normal diploids were obvious without measurements, and in fact many of the triploids were gigantic compared with any of our in-house diploid populations, domestic or wild.

DISCUSSION

Gynogenesis: The UV treatment (1400 $\mu W/cm^2$, 1.5 min, 10^8 sperm/ml, 1 mm deep) defined in this study was effective in inactivating chromosomes of *M. lateralis* sperm. In the haploid groups, none of the 300 early embryos examined were diploid. Embryos with 20 chromosomes ($n + 1$) may or may not have inherited the extra chromosome from sperm. Such a small frequency (3%) of chromosome addition could be caused by spontaneous nondisjunction of maternal chromosomes (HECHT and HECHT 1987). No chromosome fragments were observed as suggested by previous studies (XU *et al.* 1990; GUO *et al.* 1993).

Blocking PB2 restored diploidy in the gynogenetically activated eggs and also increased their survival. Allozyme inheritance in gynogens is being examined, and for 12 polymorphic loci examined so far, all paternal alleles were absent in all gynogens from all three replicates, suggesting that the gynogenesis obtained in this study is very "clean" (X. GUO and S. K. ALLEN, manuscript in preparation). The success of gynogenesis was suspected even before the electrophoretic confirmation. First, survivors (diploid) were found only in CB-treated groups, not in haploid controls. Second, 2ng survivors were all female, contrasting to the normal sex ratio in the control groups. In fish such as salmonids and tilapia, gynogenetic diploids were also all females (CHOURROUT and QUILLET 1982; MAIR *et al.* 1991)

Compared with normal diploids and triploids, the survival of gynogenetic diploids was low (0.7%). Reduced survival is common for gynogenetic fish and mollusks, and it is commonly believed to be caused by inbreeding depression (SCARPA 1985; MA 1987; XU *et al.* 1990; FUJINO

et al. 1990). On the other hand, there were several indications that inbreeding depression is not a major cause for the reduced survival. First, gynogenesis and androgenesis using inbred strains did not lead to significant improvement on survival (SCHEERER *et al.* 1986; GUO *et al.* 1993). Secondly, because of high recombination frequencies, the level of inbreeding in meiosis II gynogens was low (fixation index = 0.26), similar to that of a sister-brother mating (GUO and GAFFNEY 1993). Alternatively, GUO *et al.* (1993) suggested that damaged DNA fragments from sperm irradiation can potentially be a cause for the reduced survival.

Overall, there was no significant difference in growth measurements between gynogens and normal diploid females, suggesting again that inbreeding depression is minimal for meiosis II gynogens. On the other hand, the significant interaction between gynogenesis and replicates (or females) indicated that the performance of gynogens might be a function of the maternal genome. In the Pacific oyster, gynogenetic diploids were either smaller than or not different from diploid controls (GUO and GAFFNEY 1993).

Sex determination: The fact that gynogenetic diploids were all female strongly suggests that, in *M. lateralis*, females are homogametic (*XX*). Also the fact that no intersex individuals were found among triploids suggests that the maleness-gene is dominant, so that *XXX* is female, and *XXY* is male. Therefore, we propose that sex in *M. lateralis* is determined by a *XX*-female, *XY*-male mechanism with a dominant maleness gene(s), similar to that in mammals and other vertebrates. Under such a system, sex determination in triploids induced by blocking PB2 should be unaffected. The slightly biased sex ratio of triploids observed in this study could be caused by differential mortality of triploid males and females, or sampling errors. Previous studies on the karyotype of *M. lateralis*, however, found no evidence for dimorphic sex chromosomes (MENZEL 1968; WADA *et al.* 1990). Sex determination in mollusks is largely unknown. In the American oyster, it has been proposed that sex is determined by multiple loci (HALEY 1977, 1979). In the soft-shell clam, an *X*:autosome determination, similar to that in Drosophila, has been proposed based on the presence of possible intersexes and absence of males among triploids (ALLEN *et al.* 1986). The diverse sex determination among mollusks, if true, may limit the generality of *M. lateralis* as a model species.

It is clear that there were differences in growth between two sexes in *M. lateralis*, although incomplete maturation encountered in this study prevented a more definitive analysis. If males are larger than females in commercial species (such as hard clam and Manila clam), the creation of all male populations through androgenetic *YY*-males should be useful.

Polyploid gigantism: Triploid *M. lateralis* produced in this study were significantly larger than normal diploids, which agrees with a previous study in the same species (RUPRIGHT 1983). Besides *M. lateralis*, triploid mollusks were significantly larger than diploids in almost all species studied, including the American oyster (*Crassostrea virginica*) (STANLEY *et al.* 1984), bay scallop (*Argopecten irradians*) (TABARINI 1984), the Pacific oyster (*Crassostrea gigas*) (ALLEN and DOWNING 1986), the scallop (*Chlamys nobilis*) (KOMARU and WADA 1989), the pearl oyster (*Pinctada martensii*) (JIANG *et al.* 1991) and the scallop (*Chlamys farreri*) and the oyster (*Crassostrea rivularis*) (R. C. WANG, personal communication). Triploids were not significantly different from their diploid controls in the soft-shell clam (*Mya arenaria*) (MASON *et al.* 1988), and in one of the studies of American oyster (STANLEY *et al.* 1981). In the hard-shell clam (*Mercenaria mercenaria*), triploids were smaller than their diploid controls (HIDU *et al.* 1988).

Two hypotheses have been proposed to explain increased body size in triploid mollusks. The first is the heterozygosity hypothesis, proposed by STANLEY *et al.* (1984), stating that the increased body size in meiosis I triploids (produced by blocking PB1) was caused by increased heterozygosity. This hypothesis may also explain differences between diploids and meiosis II triploids because theoretically, all triploids (either from meiosis I or meiosis II inhibition) are more heterozygous than diploids (ALLENDORF and LEARY 1984). Supporting the heterozygosity hypothesis, meiosis I triploids were usually larger than meiosis II triploids and normal diploids (STANLEY *et al.* 1984; JIANG *et al.* 1991; BEAUMONT and KELLY 1989). The second hypothesis, originating from finfish work (PURDOM 1972), argues that the increased body size in triploids is caused by energy reallocation from gametogenesis to growth due to the sterility of triploids. Supporting the energy reallocation hypothesis, triploid Pacific oysters were larger than diploids only after spawning (ALLEN and DOWNING 1986). In other studies, energy reallocation was suggested because triploids grew faster than diploids around the time of sexual maturation (TABARINI 1984; JIANG *et al.* 1991).

We agree that both heterozygosity and energy reallocation may contribute positively to the overall performance of triploids. However, we hypothesize that they are not the primary causes for the increased body size in triploids. First, the heterozygosity hypothesis was proposed under the assumption that meiosis I triploids are more heterozygous than meiosis II triploids (STANLEY *et al.* 1984). This assumption is true only when the recombinant frequency is lower than 0.67 (GUO *et al.* 1992). A preliminary study in the Pacific oyster estimated that the average recombinant frequency (r) over seven loci was 0.74 (GUO and GAFFNEY 1993), suggesting that the heterozygosity of meiosis I triploids ($1 - r/2$) was lower than that of meiosis II triploids (r). Other studies found a lack of correlation between heterozygosity and growth rate in meiosis I and II triploids (JIANG

et al. 1991; MASON *et al.* 1988). Within natural diploid populations, the correlation between heterozygosity and growth rate is weak, although generally (but not always) positive (GAFFNEY 1990). It is hard to imagine that increased heterozygosity can increase the body size of triploids by 72% in *M. lateralis* (this study), 27–58% in *Pinctada martensii* (JIANG *et al.* 1991), 36% in *Argopecten irradians* (TABARINI 1984), 32–59% in *Chlamys nobilis* (KOMARU and WADA 1989), and 81% in *Chlamys farreri* (R. C. WANG, personal communication). Second, the energy reallocation hypothesis can not explain findings where triploids were significantly larger than diploids before sexual maturation (JIANG *et al.* 1991; R. C. WANG, personal commnication). Also in this study, triploids were larger than diploids at 30 days PF, long before sexual maturation (data not presented here). Even during maturation, it is difficult to understand how reallocation of metabolic energy (from gonad to somatic tissue) could lead to a net increase in body size, unless diploids spawned and triploids did not. In this study, neither diploids nor triploids had spawned at 3 months of age (clams were reared inside the laboratory and monitored daily). Clearly, the energy reallocation hypothesis is not sufficient to account for the increased body size in triploids observed in this and other studies.

In view of the difficulty of the existing hypotheses to adequately account for increased growth in triploid mollusks, we would argue that the increased body size in triploid mollusks is caused by a polyploid gigantism, due to the increased cell volume and a lack of cell number compensation. Polyploid gigantism was probably dismissed early in mollusk work because of the assumption that animals in general display a reduction in cell number with increasing cell size. With our data and upon further reflection, we think gigantism warrants another look.

Polyploid gigantism is a common feature in plants (BLAKESLEE 1941; STEBBINS 1956) and has also been observed in some invertebrates such as the brine shrimp (*Artemia salina*), the bagworm moth (*Solenobia* sp.), the isopod *Trichoniscus elisabethae* and Drosophila (reviewed by Fankhauser 1945). In higher animals such as fish and amphibians, however, the increased cell volume in polyploids is often compensated for by a reduction in cell numbers so that the overall size of organs was unchanged (FANKHAUSER 1945; BENFEY and SUTTERLIN 1984). We hypothesize that, in polyploid *M. lateralis* and possibly all mollusks, the cell number reduction is absent, producing polyploid gigantism. Polyploid gigantism may not be apparent in all organs (FANKHAUSER 1945). In bivalves, the adductor muscle seems particularly subject to polyploid gigantism. Adductor muscles of triploids were 73% larger than those of diploids in the bay scallop (TABARINI 1984), 96% larger in the scallop (*Chlamys farreri*) and 198% larger in the oyster (*Crassostrea rivularis*) (R. C. WANG, personal communication). In our experience with Pacific oysters, the adductor muscles of triploids are visibly larger than those of diploids, and the adductor muscle of tetraploids were extraordinarily (or abnormally) large (GUO and ALLEN 1994b and unpublished). Interestingly, since the adductor muscle is the site of at least some glycogen storage in bivalves, its increased size has also been seen as an indicator of energy reallocation (TABARINI 1984).

The expression of polyploid gigantism can be affected by environmental factors, which may explain the failure to recognize polyploid gigantism in previous studies in mollusks. One of the factors, we speculate, may be nutrition. Because triploid cells are larger, they may need more nutrients to grow and divide. In an environment where food supplies are limiting, triploids may not be larger than diploids before sexual maturation. When diploids attain sexual maturity, triploids may continue to grow and surpass diploids, masking any affect of gigantism. In more productive waters, triploid Pacific oysters often grow faster than diploids even before diploids have sexually matured (DAVIS 1989; K. COOPER, personal communication). In this study, several factors may have contributed to our finding of polyploid gigantism. First, food was not limiting. We fed experimental clams daily from algal cultures (*Isochrisis galbana* and *Chaetoceros calcitrans*) maintained in our lab. Second, neither diploid nor triploid clams had spawned prior to sampling.

Separation of the heterozygosity, energy reallocation and polyploid gigantism hypotheses is important for future research and application of polyploidy in mollusks. It is apparent that heterozygosity and energy reallocation hypotheses are insufficient, and the polyploid gigantism hypothesis needs to be confirmed by direct studies on cell size, cell number and organ size in diploids and triploids.

Fecundity of triploids: The relative fecundity of triploids observed in this study is surprisingly high, 59% for females and 79% for males. That fecundity in triploids was so high and gigantism was still apparent strengthens our hypothesis over the energy reallocation hypothesis. In the Pacific oyster, the relative fecundity of triploid females was estimated to be only 2% (GUO and ALLEN 1994a). The production of large numbers of gametes by triploids warrants further studies on their sterility.

We are grateful to AMY TAYLOR for assistance in larval and nursery cultures and in the laboratory, and to ANN E. ARSENIU for general lab assistance. An anonymous reviewer offered constructive suggestions on data analysis. This is New Jersey Agricultural Station Publication No. D-32100-2-94 and Contribution No. 94-04 of the Institute of Marine and Coastal Sciences, Rutgers University, supported by U.S. Department of Agriculture/CSRS/OGPS 92-37203-7766 to S.K.A. and X.G.

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Communicating editor: R. E. GANSCHOW