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Induced triploidy in the soft-shelled clam *Mya arenaria*: energetic implications

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Abstract

Given that triploid adult bivalves reportedly grow larger and faster than their diploid siblings, such differences should be traceable to variation in energy allocation. In one proposed mechanism, retarded gametogenesis found in triploid adults may allow them more energy for somatic growth. Another hypothesis states that triploids are more heterozygous; increased heterozygosity has been positively correlated with enhanced growth. Juvenile soft-shelled clams, *Mya arenaria*, were treated with cytochalasin B to induce triploidy and examined with respect to components of a balanced energy equation ($C = P + R + E$). The variables measured were oxygen uptake ($\dot{V}_{O_2}$), filtration rate (FR), dry tissue weight, shell length, shell height and shell inflation. Energy budgets were constructed and diploid and triploid groups compared. Few significant differences were found between diploid and triploid juvenile clams with respect to energy budget components. However, at seven loci assayed electrophoretically the triploid individuals were nearly twice as heterozygous as their diploid siblings. Moreover, triploid variances were less than diploid variances for every variable measured. Increased heterozygosity has been correlated with the decreased variance of morphological parameters. This study is believed to be the first to show decreased variance of physiological properties as well as morphological characters. Overall the data clearly indicate that energy allocation in juvenile *M. arenaria* is not related to ploidy.

Introduction

Induced triploidy is currently being evaluated as a means of enhancing production of commercially valuable bivalve species (Allen et al. 1982, Stanley et al. 1984, Tabarini 1984, Allen and Downing 1986). Polyploid organisms are commonly sterile due to problems associated with homologous chromosome pairing during meiosis, thus sterility is advantageous in that energy not utilized for reproduction may be available for somatic growth (Gabbott 1975, Stanley et al. 1981), thereby resulting in larger individuals. An added advantage to possessing an extra set of chromosomes is that it appears to increase heterozygosity. Individuals with greater heterozygosity typically show superior growth rates, increased size and generally greater developmental stability (Lerner 1954, Mitton and Grant 1984). Stanley et al. (1984) reported an increase in heterozygosity only in those triploid American oysters, *Crassostrea virginica*, in which Meiosis I had been blocked but not Meiosis II. Timing and duration of the cytochalasin treatment determined whether Meiosis I or Meiosis II was inhibited.

There is evidence that triploidy increases heterozygosity in *Mya arenaria* as well. Diploid and putative triploid individuals were assayed cytogenetically and electrophoretically at 9 to 12 months of age (Allen et al. 1982). At five polymorphic enzyme loci, triploidy was readily apparent and individuals were identified on the basis of distinct banding patterns. In addition, triploids had a modal chromosome count of $3N = 50$ (normal diploids had a modal $2N = 34$).

Upon inducing triploidy in *Crassostrea virginica*, Stanley et al. (1981) found that both treated and untreated groups averaged the same size after 8 mo. However, as 3-yr olds, triploid oysters had 40% more total volume and 12% greater shell height than their diploid siblings. Triploid bay scallops, *Argopecten irradians*, showed 73% greater adductor muscle weight than their diploid counterparts. Triploid scallops also exhibited significantly greater shell inflation, total body tissue and glycogen levels but significantly retarded gametogenesis (Tabarini 1984).

Energy budget components have been measured for a wide variety of bivalves, e.g. the American oyster (Langefoss and Maurer 1975), sea scallop (Ehinger 1978), blue mussel (Bayne 1976, Nielsen 1985) and surf clam (Goldberg 1985).

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Several reviews (Bayne 1976, Conover 1978, Bayne and Newell 1983) have integrated much of the experimental work on molluscan energetics. To date, there have been no studies on the energetics of triploid bivalves. We assume that, given equal food intake, any measured differences in growth between diploid and triploid individuals must be related to differential allocation of ingested energy. This study tested the null hypothesis that variables related to energy allocation specifically the rate of oxygen consumption ($\dot{V}_{O_2}$), filtration rate (FR), shell length, shell height, shell inflation and tissue dry weight, were the same in diploid and triploid juvenile soft-shelled clams. In addition, we tested the assumption that triploid juvenile clams are more heterozygous than their diploid siblings.

Materials and methods

A hatchery population of adult *Mya arenaria* of unknown heterozygosity were spawned in June 1984. Gametes from at least three female and eight male clams were mixed and the fertilized eggs immediately treated for 15 min with cytochalasin B to induce polyploidy (Allen et al. 1982). Theoretically, treatment within the first 15 min after fertilization blocks Meiosis I whereas the next 15-min period (15 to 30 min after fertilization) inhibits Meiosis II. Cytochalasin B, a fungal metabolite, has been shown to inhibit cytokinesis (Longo 1972, Defendi and Stoker 1973). Our intent was to establish both the treated triploid group and a treated diploid control and to maintain them under nearly identical conditions; an untreated control was not evaluated due to possible "container" effects.

After 30 d, all juveniles were transferred to stacked trays and exposed to flowing, unfiltered seawater at ambient temperatures. Data were collected at ca. 2-mo intervals and designated Group A, Group B and Group C. Individuals were acclimated to 18 °C under constant conditions immediately prior to the experimental period. Data for each group were analyzed separately due to differences in experimental design (Table 1).

For all groups, clams of 10 to 20 mm length were randomly chosen and each individual measured for length (anterior to posterior margin), height (hinge to ventral margin) and inflation (width of closed right and left valves) to the nearest 0.1 mm using vernier calipers (see Table 1 for measured shell length range). Clams were fed either the alga *Isochrysis galbana* or *Chaetoceros* spp. daily during the acclimation period.

A subsample of the treated population was sacrificed to determine ploidy using flow cytometry (modified after Chaiton and Allen 1985). Flow cytometric analysis of adult bivalve molluscs was used in this study and others (see Stanley et al. 1984, Tabarini 1984) with unequivocal results. Of the treated clams, 44% were found to be triploid. The ploidy of individual clams was determined by the same method upon completion of the experiment for each group.

Oxygen consumption rates ($\dot{V}_{O_2}$) were determined using a Radiometer Blood Gas Analyzer BMS 3 MK2 and a PHM pH/Blood Gas Monitor. Each clam was placed inside a 10-cc glass syringe containing previously well-aerated, 0.3 μm-filtered seawater (Laughlin et al. 1979). This procedure did not appear to disturb the clams but syringes were monitored visually and those trials in which clams did not normally extend their siphons were discarded. After 1 h, a sample of 500 μl from each syringe was injected into the common cuvette of the blood gas analyzer and the depression in O_2 content recorded after 2 min. A control (syringe without clams) was run with every trial. Results are expressed as mean μl O_2 h^{-1} $individual^{-1}$ of 2 to 4 replicate trials.

Feeding trials were run immediately following the respiration trials on the same individuals. Clams were placed individually in 100 ml of an initial concentration of at least 9.2×10^3 but not more than 1.7×10^4 cells ml^{-1} of *Isochrysis galbana*. Solutions were filtered prior to the addition of pure *I. galbana*. Chambers were incubated at the experimental temperature and stirred gently and continuously. The decrease in particle concentration was measured after 0.5 h using a Coulter Counter ZM with 140 μm aperture and Channelyzer. Each count was taken three times and averaged. Filtration rates (FR) were calculated using the method of Coughlan (1969).

Total production of shell and somatic tissue for each group was taken to be total length (mm) and total tissue dry

Table1. *Mya arenaria*. Experimental condition for three groups of juveniles used in energy budget determinations

		Group		
		A	B	C
Total length of experiment (d)		11	22	24
Experimental temperature (°C)		18	18	20
Age of clams (d)		274	335	408
Measured shell length range (mm)	(2N)	11.7 to 19.3	10.9 to 19.4	11.3 to 19.2
	(3N)	12.0 to 18.9	11.1 to 18.6	13.5 to 18.2
Period of acclimation to experimental temp. (d)		21	7	>40
Period of starvation prior to respiration trials (h)		72	24–48	4
Period of starvation prior to feeding trials (h)		120	48	4

weight (mg) respectively at the completion of the experiments. These values were divided by the number of days of growth to obtain a rough estimate of shell production in μm day^{-1} and somatic tissue production in μg day^{-1}. Organic matrix in bivalve shell is generally a small proportion (< 5%) of total production (Griffiths and King 1979, Shumway and Newell 1984) and was therefore excluded from the energy budget calculations.

To compute a balanced energy budget, we used the equation

$$C = P + R + E,$$

where

C is the energy equivalent of food ingested,
P the sum of energy incorporated as growth,
R the energy equivalent of metabolic heat losses and
E the rejecta, i.e., the combined energy loss in feces and urine

Rejecta, E, was estimated by difference from the equation $E = C - (P + R)$. Assuming excretion (U) to be negligible (Hughes 1970, Bayne 1976, Berry and Schleyer 1983), absorption may be calculated $A = P + R$. Absorption efficiency (%) was determined by the equation: $\% = [(P + R)/C] \times 100$.

Components for each individual were converted into both calories and joules h^{-1} g dry weight^{-1} using the following conversion factors:

1 ml O_2 = 4.83 cal = 20.21 J (Crisp 1971)
0.0235 ng = 1 cell *Isochrysis galbana* (Epifanio and Ewart 1977)
1 mg dry weight *I. galbana* = 5.609 cal = 23.47 J (Ehinger 1978)
1 mg bivalve tissue dry weight = 4.79 cal = 20.04 J (Thayer et al. 1973)

For electrophoresis, adductor muscles of 22 diploid and 24 triploid clams from Group C were examined at seven enzyme loci: adenylate kinase (AK; 2.7.4.3), alanine aminotransferase (ALAT; 2.6.1.2), carboxylesterase (EST-D; 3.1.1.* – an isozyme unique to the substrate 4-methylmelliferyl acetate), isocitrate dehydrogenase (IDH; 1.1.1.42), phosphoglycerate kinase (PGK; 2.7.2.3), phospho-gluconate dehydrogenase (PGDH; 1.1.1.44), and triose-phosphate isomerase (TPI; 5.3.1.1). AK, IDH, PGDH, PGK, and TPI were resolved with an amine-citric acid buffer with 0.01 M EDTA (Clayton and Tretiak 1972), ALAT using a Tris-boric-EDTA buffer (Boyer et al. 1963) and EST-D using a phosphate buffer (Wolf et al. 1970). Staining protocols of Aebersold et al. (1987) were used for all enzymes. The seven loci were scored as either homozygous or heterozygous and the average proportion of heterozygous loci per individual was estimated by averaging, over all loci, the proportion of heterozygous individuals at each locus (Table 2).

Shell length, shell height and shell inflation, $\dot{V}_{O_2}$ and FR were each regressed as a function of dry weight for diploid and triploid clams within each group. Linear regression lines were calculated using both untransformed and logarithmically transformed data. The slopes of diploid and triploid regression lines were compared by the homogeneity-of-slopes model for one-way analysis of variance (ANOVA: SAS User's Guide 1985). The sources of variation for each model were dry weight, ploidy and the interaction of weight and ploidy. Diploid and triploid mean values for all parameters were compared using Student's t-test ($p < 0.05$) within group only, i.e., no statistical comparisons were made between Groups A, B and C. Differences between diploid and triploid variances were tested using the variance ratio test (Zar 1974).

Results

Morphological and physiological data were collected from 38 juvenile *Mya arenaria* in Group A, 46 in Group B, and 24 in Group C, for a total of 108 juvenile clams in this study. By flow cytometric analysis, it was determined that 45% of the experimental individuals were triploid. The mean proportion of heterozygous loci for diploids was 0.11 and for triploids, 0.21 (Table 2).

Morphological and physiological comparisons

Morphological variables, i.e., length, height and inflation, were highly correlated with dry weight for all groups ($r^2 > 0.61$). The intercepts and slopes for length, height and inflation were quite similar among groups. In contrast, the physiological variables, $\dot{V}_{O_2}$ and filtration rate, had much lower correlation coefficients overall as well as higher variability between groups. Filtration rates were especially variable within and between groups. For all three groups, ANOVA revealed no significant effects of ploidy or the interaction of weight and ploidy for any of the variables.

The results of Student's t-tests for mean values of measured variables in diploid and triploid clams revealed a total of only 5 significant differences at $p < 0.05$ out of 24 comparisons (Table 3). For Group A, inflation was significantly greater in diploids than triploids. For other comparisons in Group A, length, dry weight, FR, shell production and tissue production of triploids were only slightly greater than diploids. Inflation and filtration rate means of triploids in Group B (Table 3) were significantly less than diploid means ($p < 0.05$) while all other mean values of triploids in Group B were slightly less than those of diploid means. Similarly, the means of most parameters in Group C triploids were somewhat less, height and dry weight being significantly less. It is notable that height, inflation and $\dot{V}_{O_2}$ were slightly or significantly less in triploids for all groups. All means for all of the variables in Group B and C triploids were less (Table 3).

Despite the lack of consistent morphological or physiological distinctions between diploid and triploid animals, one trend was apparent. The slopes of regression lines for length as a function of dry weight in triploids were steeper than those for diploids in all groups (Fig. 1). That it, for a given length the average triploid clam weighed slightly less

Table 2. *Mya arenaria*. Seven loci were examined electrophoretically in Group C diploid and triploid individuals and scored as either homozygous (ho) or heterozygous (he). Ploidy was determined by flow cytometry prior to electrophoresis. The proportion of heterozygous individuals (PHI) was calculated at each locus, and the mean proportion of heterozygous individuals was averaged over all loci

Diploid clam no.	Enzyme locus							
	AK	ALAT	EST-D	IDH	PGK	PGDH	TPI	
2	he	ho	he	ho	ho	he	ho	
7	ho	ho	ho	ho	ho	ho	ho	
9	ho	ho	ho	ho	–	ho	ho	
10	ho	ho	ho	ho	–	ho	ho	
12	ho	ho	ho	ho	–	he	ho	
13	ho	ho	ho	ho	ho	ho	ho	
15	ho	ho	ho	ho	ho	he	ho	
16	ho	ho	ho	ho	he	ho	ho	
18	ho	ho	ho	ho	ho	ho	ho	
19	ho	ho	ho	ho	ho	ho	ho	
21	ho	ho	he	ho	–	ho	ho	
22	ho	ho	he	ho	–	he	ho	
29	ho	ho	ho	ho	ho	he	ho	
49	ho	ho	ho	ho	ho	ho	ho	
51	ho	ho	he	ho	ho	he	ho	
54	ho	ho	ho	ho	ho	ho	ho	
56	ho	ho	ho	ho	ho	ho	ho	
59	ho	ho	ho	ho	ho	he	ho	
104	ho	ho	ho	ho	ho	he	ho	
105	ho	ho	ho	ho	ho	he	ho	
106	ho	ho	ho	ho	ho	ho	ho	
112	ho	ho	he	ho	ho	ho	ho	x (s.e.)
PHI	0.04	0.00	0.23	0.00	0.06	0.41	0.00	0.11 (0.06)
Triploid clam no.								
1	he	–	he	ho	–	ho	he	
3	ho	ho	ho	ho	ho	he	ho	
4	ho	ho	ho	ho	ho	ho	ho	
5	ho	ho	ho	ho	ho	he	ho	
6	ho	ho	he	ho	ho	he	ho	
8	ho	ho	ho	ho	ho	he	ho	
11	ho	ho	ho	ho	ho	he	ho	
14	ho	ho	ho	ho	ho	he	ho	
17	he	he	–	ho	ho	ho	he	
20	ho	he	ho	ho	ho	he	ho	
27	he	ho	–	ho	ho	ho	ho	
34	ho	ho	ho	ho	ho	he	ho	
39	ho	ho	ho	ho	ho	he	ho	
43	ho	ho	ho	ho	ho	he	ho	
46	ho	ho	–	ho	ho	he	ho	
47	ho	ho	ho	ho	ho	he	ho	
60	ho	ho	ho	ho	ho	he	ho	
63	he	he	–	ho	he	ho	ho	
69	he	–	–	ho	he	he	ho	
75	ho	ho	ho	ho	ho	he	ho	
86	ho	ho	ho	ho	ho	he	ho	
109	ho	ho	ho	ho	ho	he	ho	
114	ho	ho	he	ho	ho	he	ho	
118	ho	ho	–	ho	ho	he	ho	x (s.e.)
PHI	0.21	0.14	0.17	0.00	0.09	0.79	0.08	0.21 (0.10)

than its diploid sibling. Height and shell inflation were also compared but no significant trends were apparent (data not shown).

Diploid clams in Group A consumed slightly more oxygen per gram dry weight than triploids, but in Group B diploids consumed slightly less per gram dry weight (Fig. 2). The regression of $\dot{V}_{O_2}$ on dry weight was not significant for triploids in Group C. There was considerable variation among individuals with respect to FR, resulting in only one possible (Group A) diploid:triploid comparison (Fig. 3).

Variances associated with every variable in every group were less in triploids than diploids (Table 3). Results of the variance ratio test (Table 4) revealed that in 13 of 24

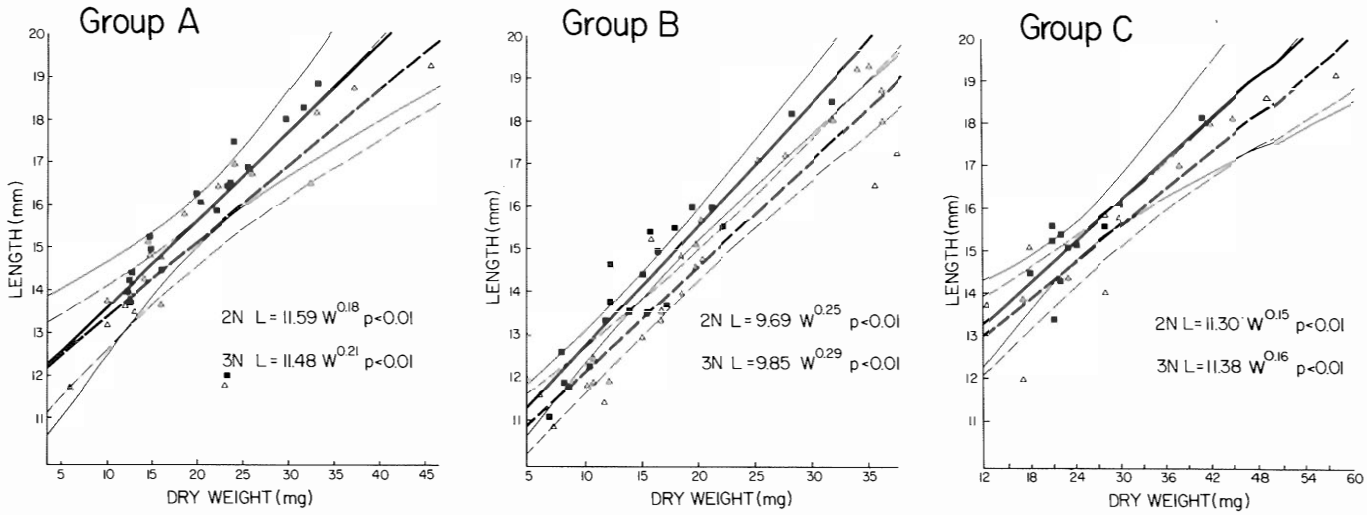

Fig. 1. *Mya arenaria.* Relationship between shell length and tissue dry weight for diploid (△) and triploid (■) juveniles. Lines represent least squares regressions ± 95% confidence intervals (solid lines = 3N, dashed lines = 2N)

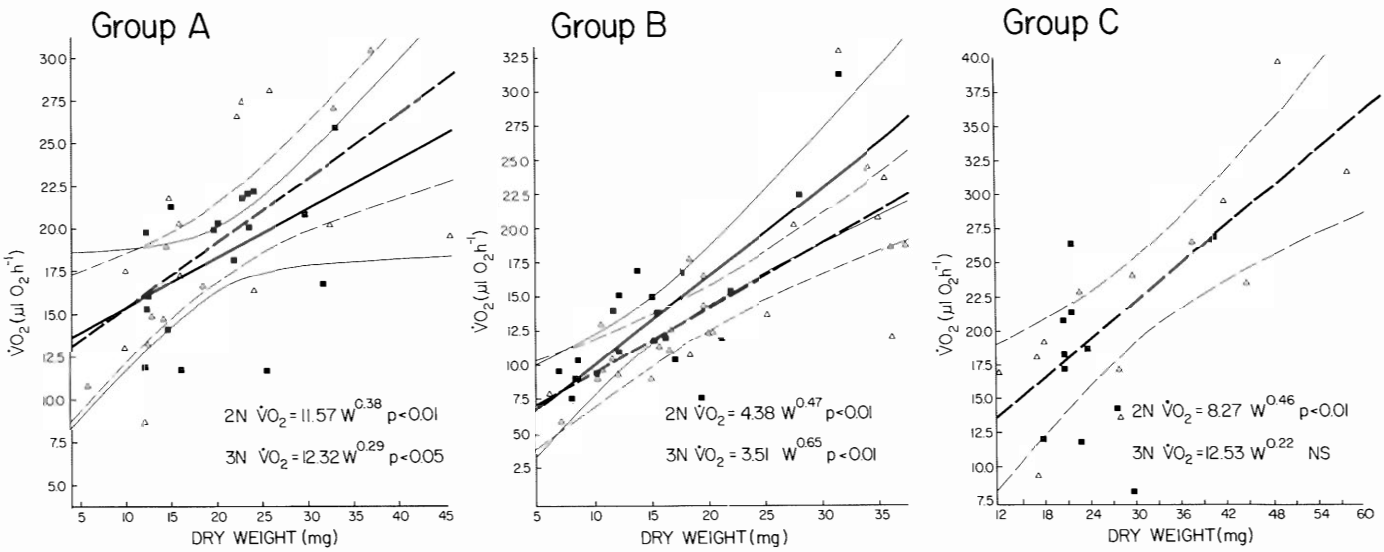

Fig. 2. *Mya arenaria.* Relationship between oxygen consumption and tissue dry weight for diploid (△) and triploid (■) juveniles. Lines represent least squares regressions ± 95% confidence intervals (solid lines = 3N, dashed lines = 2N)

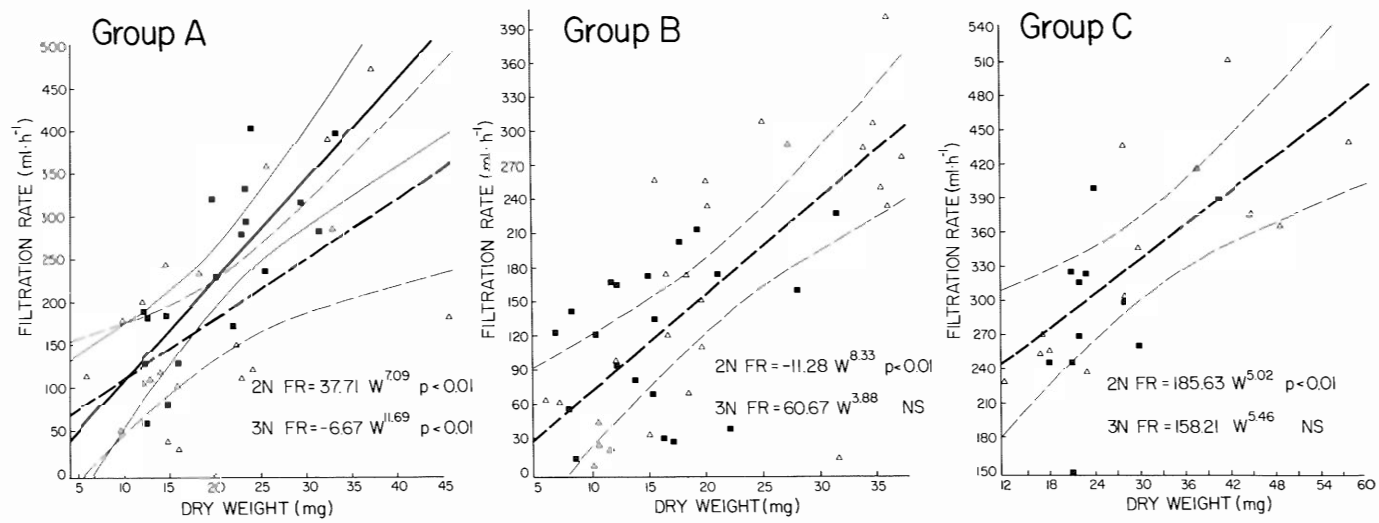

Fig. 3. *Mya arenaria.* Relationship between filtration rate and tissue dry weight for diploid (△) and triploid (■) juveniles. Lines represent least squares regressions ± 95% confidence intervals (solid lines = 3N, dashed lines = 2N)

Table 3. *Mya arenaria*. Student's *t*-test results comparing diploid and triploid juveniles with respect to eight variables. An asterisk indicates that the means are significantly different at $p<0.05$

		Group								
		A[a]			B[b]			C[c]		
		Mean	t Value	Var	Mean	t Value	Var	Mean	t Value	Var
Length (mm)	2N	15.15	1.42	4.62	14.94	1.75	7.08	15.86	3.58	5.02
	3N	15.77		3.28	14.36		4.04	15.37		1.44
Height (mm)	2N	9.41	2.04	1.72	9.23	2.36	3.10	10.19	4.08*	2.46
	3N	9.27		0.83	8.55		1.32	9.48		0.61
Inflation (mm)	2N	5.46	3.13*	0.81	5.32	2.82*	1.19	5.69	2.49	0.94
	3N	5.37		0.26	4.82		0.42	5.31		0.37
Dry wt (mg)	2N	20.05	2.34	107.33	21.09	2.33	101.00	31.15	4.94*	201.92
	3N	20.67		45.97	15.58		43.30	24.64		40.83
$\dot{V}_{O_2}$ (μl O_2 l^{-1} h^{-1})	2N	19.21	2.20	37.70	14.71	1.23	38.07	22.54	1.88	65.77
	3N	18.35		17.14	13.65		30.91	17.86		35.05
Filtration rate	2N	179.80	1.41	14 566.1	164.36	3.05*	13 271.0	341.92	1.66	8 372.3
(ml h^{-1})	3N	235.03		10 363.2	121.13		4356.0	292.84		5 046.7
Shell production	2N	55.35	0.94	58.71	44.61	0.82	60.53	38.60	0.45	31.43
(μ day^{-1})	3N	57.57		41.02	42.87		34.17	37.73		8.04
Tissue production	2N	73.18	0.22	1 359.48	62.95	1.12	865.55	76.36	1.40	1 120.0
(μg day^{-1})	3N	75.45		577.73	46.51		367.02	60.38		223.1

[a] 20 2N and 18 3N individuals
[b] 26 2N and 20 3N individuals
[c] 13 2N and 11 3N individuals

Table 4. *Mya arenaria*. Calculated F values of one-tailed variance ratio test for difference between two variances

		Group		
		A	B	C
df	2N	19	25	12
	3N	17	19	10
Length (mm)		1.41	1.75	3.49*
Height (mm)		2.07	2.35*	4.03*
Inflation (mm)		3.12*	2.83*	2.54
Dry weight (mg)		2.33*	2.33*	4.95**
$\dot{V}_{O_2}$ (μl O_2 l^{-1} h^{-1})		2.20	1.23	1.88
Filtration rate (ml h^{-1})		1.41	3.05**	1.66
Shell production (μm day^{-1})		1.43	1.77	3.91*
Tissue production (μg day^{-1})		2.35*	2.36*	5.02**

* $=P<0.05$
** $=P<0.01$

comparisons, triploid variances were significantly lower ($p<0.05$).

The completed energy budgets presented contradictory, although not significantly different patterns with respect to Groups A, B and C (Table 5). Group A triploids consumed somewhat more energy (i.e., food) than Group A diploids, but Group B and C diploids consumed the greater amount in their respective groups. For respiration, *R*, the situation was exactly reversed. Group A triploids expended slightly less energy in oxygen consumption than their diploid counterparts, but Groups B and C triploids expended a little more energy per gram dry weight.

Discussion

Although energetic differences between diploid and triploid juvenile *Mya arenaria* were not demonstrated in the present study, a number of important observations were made. The average triploid clam in all three groups weighed slightly less than the average diploid at a given length. In addition, as a triploid clam increased in body weight, its shell tended to be less inflated than that of a diploid sibling of equal size. These measurements were somewhat surprising in the light of previous studies (Stanley et al. 1981, 1984, Tabarini 1984). In fact, triploid scallops were found to have significantly greater shell inflation than diploids (Tabarini 1984).

The majority of triploid means were less than the diploid means. This may be indicative of how triploidy affects young individuals and should be further investigated. Thus, although all individuals within each group were exposed to identical conditions, on the average the diploids appeared to be more "robust". Allen et al. (1982) reported that cytochalasin B retards larval development in *Mya arenaria* up to Day 11. It seems reasonable to assume that triploids per se poses energetic and metabolic difficulties on a cellular level not experienced by normal diploids.

Table 5. *Mya arenaria*. Mean values of energy budget components for diploid and triploid juveniles (10 to 20 mm shell length). Energy units are given in joules h^{-1} per gram dry wt. calories h^{-1} g^{-1} are given in parentheses

N	Dry tissue wt (mg)	Ingested ration (C)			Respiration rate		Production (P)		Absorption (A)	
		Cells $\times 10^3$ per ml	Filtration rate	(J h^{-1} g^{-1})	(μl O_2 h^{-1})	(J h^{-1} g^{-1})	μg dry tissue per day	J h^{-1} g^{-1}	P+R J h^{-1} g^{-1}	$\% = \left(\frac{P+R}{C}\right) \times 100$
Group A										
2N 20	20.1	7.18	179.55	44.67 (10.68)	19.21	19.32 (4.62)	73.18	3.04 (0.73)	22.36 (5.34)	52.7
3N 18	20.8	8.13	235.03	56.78 (13.57)	18.35	17.83 (4.26)	75.45	3.03 (0.72)	20.86 (4.99)	41.5
Group B										
2N 26	21.1	7.46	164.37	42.73* (10.45)	14.71	14.09 (3.37)	62.95*	2.49 (0.60)	16.58 (3.96)	36.3
3N 20	15.6	6.24	121.13	32.76 (7.83)	13.65	17.68 (4.23)	46.51	2.49 (0.60)	20.17 (4.82)	48.7
Group C										
2N 13	31.2*	11.23	341.93	67.87 (16.22)	22.63	14.60 (3.49)	76.36	2.04 (0.49)	16.64 (3.98)	25.5
3N 11	24.6	8.75	292.84	58.51 (13.98)	17.71	14.67 (3.51)	60.38	2.05 (0.49)	16.72 (4.00)	27.0

* Significantly different within group at $P < 0.05$

Estimated filtration rates ranged from 121 to 292 ml h^{-1} individual^{-1}, consistent with reported values for *Mya arenaria* (Allen 1962) and other bivalve species (Newell 1979, Gerdes 1983). Ingested ration was calculated on a weight-specific basis; overall, energetic intake per gram dry weight was very consistent between groups (Table 4). Likewise, average values for $\dot{V}_{O_2}$ were very comparable between groups and fall within the range reported for other molluscs (Dame 1972, Vahl 1973, Hamburger et al. 1983). Metabolic losses vary considerably under different conditions and feeding regimes (Bayne and Newell 1983); we attempted to minimize these losses by standardizing conditions within each group. On average, 86.6% of the absorbed ration was expended on respiration in this study.

Approximately 13.3% of the absorbed ration was incorporated into somatic tissue. Although C and R are relatively straightforward measurements, the production term P is often difficult to measure because of the complex balance between somatic growth and reproductive growth. The production term P varies widely in individuals in response to both intrinsic and extrinsic factors. Estimates of P are further complicated by seasonal and sexual differences in energy allocation. Our values for P may be underestimated, because they do not include organic matter that is incorporated into the shell. Estimates of organic matter in bivalve shell range from 1% in *Mulinia lateralis* (Shumway and Newell 1984) to 4.3% in *Mytilus edulis* (Jorgensen 1976).

Juveniles were used in this study both to simplify the measurement of production and to determine if energetic differences exist prior to sexual maturation. To the best of our knowledge, the 408-d-old animals had not initiated gametogenesis. Maximum shell length was approximately 35 mm at the end of this study; *Mya arenaria* normally mature at about 5 cm shell length. At the age of 3 yr, this same stock of triploid individuals exhibited evidence of abnormal and reduced gametogenesis.

Absorption efficiencies, presented here are somewhat low (38.6%) a range of 60 to 70% being considered normal for laboratory individuals fed cultured algae (Newell 1983). However, Conover (1978) reviewed the literature for suspension and deposit feeders and found efficiencies as low as 25.8% and as high as 79.9%. Our attempts to calculate absorption efficiency directly with the ash ratio method of Conover (1966) were unsuccessful.

An important and interesting finding was that all triploid variances were less than diploid variances (Table 3). Lerner (1954) stated that higher levels of heterozygosity confer enhanced developmental homeostasis resulting in lower levels of phenotypic variance. Empirical support for this view was provided by a study of six polymorphic enzyme-loci in the monarch butterfly in which the heterozygotes had smaller variances for morphological characters when compared to homozygotes at the same locus (Eanes 1978). Similar results were reported for killifish (Mitton 1978) but not for plaice, *Pleuronectes platessa* (McAndrew et al. 1982). Two other studies have reported results for bivalves similar to those of Eanes (1978) and Mitton (1978). The variance in growth rate, expressed as the coefficient of

variation, decreased significantly with increasing heterozygosity in both *Crassostrea virginica* (Zouros et al. 1980) and *Mytilus edulis* (Koehn and Gaffney 1984). Based on the literature, the reduced variance found for all the variables in the present study strongly suggests that it is related to increased heterozygosity (Mitton and Grant 1984, Mitton and Koehn 1985). The present report is believed to be the first to show decreased variance of physiological properties as well as morphological characters with an increase in heterozygosity.

Increased heterozygosity has been positively correlated with increased body weight and growth rate in *Crassostrea virginica* (Singh and Zouros 1978; Zouros et al. 1980), *Crassostrea gigas* (Fujio 1982) and *Macoma balthica* (Green et al. 1983). There is also substantial evidence that more heterozygous individuals are physiologically more efficient (Koehn and Shumway 1982, Rodhouse and Gaffney 1984, Garton et al. 1984, Koehn and Gaffney 1984). According to Koehn and Shumway (1982), higher growth rates in more heterozygous American oysters were achieved by a lower cost of routine metabolism reflected by a lower rate of oxygen consumption. In the single report correlating energy budget parameters with heterozygosity, Garton et al. (1984) found a positive relationship between observed growth and multi-locus heterozygosity and a negative relationship between routine metabolic costs and heterozygosity in the coot clam *Mulinia lateralis*.

No correlation between growth and heterozygosity has been reported for several bivalve species: *Pecten maximus* (Wilkins 1978, Beaumont et al. 1985), *Mytilus edulis* (Beaumont et al. 1983), *Mercenaria mercenaria* (Adamkiewicz et al. 1984), *Placopecten magellanicus* (Foltz and Zouros 1984) and *Mulinia lateralis, Spisula solidissima* and *Crassostrea virginica* (Gaffney and Scott 1984). Koehn and Gaffney (1984) suggested that the disparity may lie in the experimental design, i.e., a positive correlation is not seen where there has been only limited genetic sampling of the natural genetic variation.

Some of the variability between groups may be explained by the experimental conditions. A major difference between Groups A, B and C was the age of the individuals and its effect on their size. Group A was 9 to 10 mo, Group B 11 to 12 mo and Group C 14 to 15 mo of age, yet all experimental clams were chosen randomly in the same size range. One might hypothesize that the youngest group consisted primarily of fast growers and the oldest group of slow growers. This implies differing levels of heterozygosity irrespective of ploidy. However, since our objective was to determine differences between diploid and triploid within a particular group, this was not deemed a problem.

One of the disadvantages in a study of this nature is the amount of "noise" or randomness inherent in biological systems. According to proponents of the adaptability theory, the variability of data in living systems is at least as fundamental as any other property of those systems. The adaptability theorist views variability as having functional significance in and of itself, whereas the statistician hopes to define, with a certain degree of confidence, some relationship which the variability is presumed to mask (Conrad 1983). Conrad also points out that the variabilities of biological systems are essential to their integrities in different but equally important ways and, as such, should not be considered merely extraneous.

Our data clearly demonstrate that energy utilization in juvenile soft-shelled clams is not directly related to ploidy level, per se. We also have no evidence that the increased heterozygosity found in triploid animals has any effect on the way in which they allocate energy, although it is often stated that heterozygous individuals grow larger, faster or are otherwise more robust as a result of increased genetic diversity (see Mitton and Grant 1984). For example, Rodhouse et al. (1986) concluded that more heterozygous *Mytilus edulis* allocate more energy to somatic growth during early life (Rodhouse et al. 1986). Why then do the triploid juvenile clams show no differences despite electrophoretic evidence presented here that they are indeed more heterozygous?

We suggest two possible explanations for our results: First, it may be that the greater growth in adult triploid bivalves is due primarily to the blockage of gametogenesis and consequent reallocation of energy to somatic tissue rather than to fundamental changes in physiological and metabolic processes. Gametogenesis was reported to be severely retarded in triploid *Mya arenaria* (Allen et al. 1986) and triploid *Argopecten irradians* (Tabarini 1984). Another possible explanation is that in our study, the relationship between heterozygosity and growth was masked by the particular design of our experiment. Koehn and Gaffney (1984) found that all studies which involved laboratory-reared individuals from a limited number of parents often failed to show a positive relationship between growth and heterozygosity. In our study, at least three females and eight males were spawned; however, all parents came from a domesticated hatchery population and juveniles were exclusively laboratory-reared. Finally, it is possible that both of the above contribute in some way and that differences between triploid and diploid are simply more easily discerned in adult bivalves.

Production of superior breeding stocks in marine organisms, using classical methodologies for genetic improvement, are lengthy and costly at present. By combining studies on energy utilization and genetics, the potential exists for producing shellfish which possess substantially increased growth rates and decreased development times. Use of induced polyploidy may prove to be a useful option for breeding superior strains, especially via increased heterozygosity, as well as increasing production potential for a given species.

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