

2-2018

## Mitotic instability in triploid and tetraploid one-year-old eastern oyster, *Crassostrea virginica*, assessed by cytogenetic and flow cytometry techniques

JT de Sousa

*Virginia Institute of Marine Science*

Standish K. Allen Jr.

*Virginia Institute of Marine Science*

BM Wolfe

*Virginia Institute of Marine Science*

JA Moss

*Virginia Institute of Marine Science*

Follow this and additional works at: <https://scholarworks.wm.edu/vimsarticles>



Part of the [Aquaculture and Fisheries Commons](#)

---

### Recommended Citation

de Sousa, JT; Allen, Standish K. Jr.; Wolfe, BM; and Moss, JA, "Mitotic instability in triploid and tetraploid one-year-old eastern oyster, *Crassostrea virginica*, assessed by cytogenetic and flow cytometry techniques" (2018). *VIMS Articles*. 1294.

<https://scholarworks.wm.edu/vimsarticles/1294>

This Article is brought to you for free and open access by the Virginia Institute of Marine Science at W&M ScholarWorks. It has been accepted for inclusion in VIMS Articles by an authorized administrator of W&M ScholarWorks. For more information, please contact [scholarworks@wm.edu](mailto:scholarworks@wm.edu).



**Mitotic instability in triploid and tetraploid one-year old Eastern oyster, *Crassostrea virginica*, assessed by cytogenetic and flow cytometry techniques**

Journal:	<i>Genome</i>
Manuscript ID	gen-2017-0173.R1
Manuscript Type:	Article
Date Submitted by the Author:	23-Oct-2017
Complete List of Authors:	Sousa, Joana; Virginia Institute of Marine Science, Fisheries Allen, Standish; Virginia Institute of Marine Sciences, Wolfe, Brittany; Virginia Institute of Marine Science, Fisheries Small, Jessica; Virginia Institute of Marine Science, Fisheries
Is the invited manuscript for consideration in a Special Issue? :	N/A
Keyword:	crassostrea virginica, aneuploidy, mitotic instability, cytogenetics, flow cytometry

SCHOLARONE™  
Manuscripts

1 **Mitotic instability in triploid and tetraploid one-year old Eastern oyster, *Crassostrea***  
2 ***virginica*, assessed by cytogenetic and flow cytometry techniques**

3

4

5 **Authors' names:**

6 **Joana Teixeira de Sousa<sup>\*</sup>, Standish K. Allen, Jr., Brittany M. Wolfe, Jessica Moss Small**

7

8

9 **Addresses:**

10 **Aquaculture Genetics and Breeding Technology Center. Virginia Institute of Marine**  
11 **Science. Gloucester Point, VA 23062, USA.**

12

13

14

15

16 **\* Corresponding author**

17 **Phone: 804.684.7896 - Fax: 804.684.7717**

18 **Email: [jtsousa@vims.edu](mailto:jtsousa@vims.edu)**

19

20

21

22

23

24

## Abstract

25 For commercial oyster aquaculture, triploidy has significant advantages. To produce  
26 triploids, the principal technology uses diploid x tetraploid crosses. The development of  
27 tetraploid brood stock for this purpose has been successful, but as more is understood about  
28 tetraploids, it seems clear that chromosome instability is a principal feature in oysters. This  
29 paper is a continuation of work to investigate chromosome instability in polyploid *Crassostrea*  
30 *virginica*. We established families between tetraploids – apparently stable (non-mosaic) and  
31 unstable (mosaic) – and normal reference diploids, creating triploid groups, as well as tetraploids  
32 between mosaic and non-mosaic tetraploids. Chromosome loss was about the same for triploid  
33 juveniles produced from either mosaic or non-mosaic tetraploids or from either male or female  
34 tetraploids. However, there was a statistically significant difference in chromosome loss in  
35 tetraploid juveniles produced from mosaic versus non-mosaic parents, with mosaics producing  
36 more unstable progeny. These results confirm that chromosome instability, as manifested in  
37 mosaic tetraploids, is of little concern for producing triploids, but is clearly problematic for  
38 tetraploid breeding. Concordance between the results from cytogenetics and flow cytometry  
39 was also tested for the first time in oysters, by assessing the ploidy of individuals using both  
40 techniques. Results between the two were non-concordant.

41

42

43

44

45 **Key words:** *Crassostrea virginica*, aneuploidy, polyploidy, mitotic instability, cytogenetics,  
46 flow cytometry

47

## Introduction

48 Polyloids, organisms having more than two chromosome sets, possess some advantages  
49 compared to diploids (Comai 2005). One of the advantages, hybrid vigor, can obtain in  
50 organisms with more than two alleles (Chen 2010). In nature, these advantages have allowed the  
51 proliferation of polyploid species, especially in plants. Polyploidy has also been exploited in  
52 plants for agricultural advantages, like heterosis, gene redundancy, and self-fertilization, but this  
53 is seldom the case in animals. An exception to polyploidy in animal breeding is oysters of the  
54 genus *Crassostrea*, which have been successfully exploited as triploids (Guo et al. 2009; Nell  
55 2002; Piferrer et al. 2009). Triploid oysters are valued for their sterility that generates several  
56 advantages for oyster culture, such as reduced gonadal development that allows for higher  
57 growth rates and superior market quality during the reproductive season (Allen 1988).  
58 Otherwise, natural polyploidy in bivalves is rare, documented in only a few species (Foighil and  
59 Thiriot-Quievreux, 1991; Lee 1999; Park et al. 2000; Petkevičiūtė et al. 2007; Thiriot-Quievreux  
60 et al. 1988).

61 For all the advantages that polyploidy can confer, there are also disadvantages. For example,  
62 during mitosis, polyploidy increases the occurrence of spindle irregularities, which can lead to  
63 the chaotic segregation of chromatids and to the production of aneuploid cells (cells with a  
64 chromosome number that is not the exact multiple of the haploid karyotype) (Comai 2005;  
65 Griffiths et al. 1999; Storchova and Kuffer 2008). Indeed, aneuploidy is frequently observed in  
66 chemically induced triploid (Wang et al. 1999) and tetraploid oysters (Guo and Allen 1994;  
67 Wang et al. 1999). For oysters, chromosome loss is not limited to aneuploidy, but also includes  
68 the loss of what appear to be entire sets of chromosomes (as principally observed through flow  
69 cytometry) to become heteroploid mosaics (herein called “mosaics”) through a process called

70 reversion (Allen et al. 1996; Zhang et al. 2010). Initial investigations into using mosaic  
71 tetraploids to make triploids concerned ramifications to commercial production, that is, would  
72 triploids produced from mosaics show evidence of chromosome loss, show evidence of  
73 decreased performance, or both – the so-called heritability of chromosome loss. Earlier work on  
74 this subject found no evidence of heritability of chromosome instability between non-mosaic and  
75 mosaic parents in triploid *Crassostrea virginica*, as measured by both flow cytometry (FCM)  
76 (Matt and Allen 2014) and chromosome counts (de Sousa et al. 2016), revealing that tetraploid  
77 mosaics seem to have little impact at least for commercial triploid production. Still at issue,  
78 however, is the implication of chromosome instability in tetraploid x tetraploid crosses.

79 FCM is the principal research tool for detecting reversion because it is highly reliable and  
80 cost-effective for every stage in the life cycle of the animal. For estimation of DNA content,  
81 FCM relies on quantitative staining of nucleic acids in the nucleus, such as with propidium  
82 iodide or 4,6-diamino-2-phenylindole (DAPI). Typically, cells from any given tissue are  
83 disaggregated, and sometimes enucleated, to create a suspension of single cells (or nuclei) in  
84 which the DNA contents are individually quantified at a high rate of speed. FCM is the  
85 technique of choice for detecting triploidy because it is fast, accurate, and can be used on a  
86 variety of tissues that can be sampled without killing the animal (Allen 1983). However, it is  
87 more difficult to detect small differences in DNA content and, consequently, the data contain  
88 little information about aneuploidy. To overcome these issues, cytogenetics (chromosome  
89 counts) can be performed. Although the technique is time consuming and involves a certain  
90 degree of expertise, it is a reliable and direct method of ploidy verification. At issue is the level  
91 of chromosome loss – undetectable with FCM – in progeny created from either mosaic or non-

92 mosaic tetraploid parents. Previous work on early embryos from tetraploid crosses examined  
93 triploid but not tetraploid crosses (de Sousa et al. 2016).

94 In the present work, the level of aneuploidy from triploid and tetraploid 1yr old juveniles,  
95 produced from both mosaic and non-mosaic male and female tetraploids, were examined using  
96 cytogenetics. The main objective was to determine if there was evidence for chromosome  
97 instability in the progeny of mosaics. To that end, we established families between mosaic and  
98 non-mosaic tetraploids with reference diploids creating triploid groups, as well as between  
99 mosaic and non-mosaic tetraploids creating tetraploid groups. In addition, a concordance  
100 between the results from cytogenetics and FCM was tested for the first time in oysters, by  
101 assessing the ploidy with both techniques in the same individuals.

102

103

## Materials and methods

### 104 Experimental population and crosses

105 Tetraploid *C. virginica* brood stock were obtained from lines propagated by the Aquaculture  
106 Genetics and Breeding Technology Center (ABC) spawned in 2012. Tetraploid oysters were  
107 opened and males and females sorted. From each tetraploid, a 4 mm<sup>2</sup> gill sample was dissected  
108 from one lamella and processed for FCM (Allen et al. 1996). Gill cells were stained in  
109 DAPI/DMSO (Allen and Bushek 1992) and analyzed on a Partec Cyflow Space flow cytometer.  
110 Samples were assessed with reference to a diploid standard (gill tissue) and expressed as mean  
111 relative DNA content along with the coefficient of variation (CV) in DNA content of the cell  
112 population. For spawners, gill samples were taken as an indication of somatic ploidy with the  
113 intention of obtaining tetraploids with only tetraploid cells apparent (herein called “non-  
114 mosaics”) and also obtaining tetraploids that had multiple ploidy types in the somatic tissue

115 (“mosaics”). Gametes from diploids were obtained from a single male or a single female,  
116 depending on the test crosses.

117 Crosses were made in July 2014. After confirmation of ploidy in parents, males and females  
118 were strip spawned using the technique outlined by Allen and Bushek (1992). We made a total  
119 of 30 families: 20 triploid families with either non-mosaic (13) or mosaic tetraploid parents (7),  
120 using both sexes, with a single reference diploid (Fig. 1). We also made 10 tetraploid x  
121 tetraploid matings between non-mosaic parents (5) and between mosaic parents (5). Only 6  
122 families from groups 2 and 7 were used for cytogenetic analysis. The crossing design is shown  
123 in Figure 1.

124

### 125 **Larval rearing**

126 Larvae were reared in 110 L tanks with continuous airflow for oxygenation and circulation.  
127 Larval tanks were kept at  $25\text{ }^{\circ}\text{C} \pm 0.9^{\circ}\text{C}$  and experienced a salinity range from 12.5 to 14 PSU.  
128 Water changes occurred every other day and were larvae fed once daily with a mixture of the  
129 microalgae *Isochrysis galbana* (clone T-ISO), *Tetraselmis chui* and *Chaetoceros muelleri*  
130 (Chagra).

131 On day 2, in order to calculate the total number of larvae in the culture and their length,  
132 larvae were isolated on a top (48  $\mu\text{m}$ ) and bottom sieve (35  $\mu\text{m}$ ). Larvae collected on the 48  $\mu\text{m}$   
133 sieve on day 2 were returned to culture until day 4, at which time they were isolated on larger  
134 and larger sieve sizes until setting started on day 17, following standard protocol. Larvae were  
135 sampled on day 8 for ploidy analysis. Number of larvae collected on sieves was estimated  
136 during each water change by counting three aliquots at appropriate dilutions. An average size  
137 was taken from 10 random larvae for each culture. The sizes and larval counts were used to



138 determine growth and survival, respectively. From day 22 to day 27, individual measurements  
139 of eyed larvae length were taken from each population collected on the harvest sieve (250  $\mu\text{m}$ ).  
140 Pediveliger larvae were set on microcultch. Spat were again sampled at 2 months old for ploidy  
141 analysis via FCM and, at that time, the seed from the 30 crosses was deployed to the field at  
142 between 5-10 mm.

143

#### 144 **Rearing in the field**

145 Seed were deployed in the Coan River, Lewisetta, Virginia in September 2014. In March 2015,  
146 eight months after setting, at approximately 25 mm shell length, seed were moved from the  
147 Lewisetta site to the York River, Gloucester Point, Virginia, where temperature ranged from 14.4  
148  $^{\circ}\text{C}$  to 30  $^{\circ}\text{C}$  and salinity ranged from 13.4 to 22.9 PSU. Due to poor survival during  
149 overwintering, not all groups were available to move to the York. From triploid families using  
150 female tetraploid parents (3F), only 4 groups remained and were deployed (1, 2, 7 and 9). From  
151 triploid families using male tetraploid parents (3M), all groups were deployed. Finally, from the  
152 tetraploid families (4N), 8 groups remained and were deployed (1, 2, 4, 5, 7, 8, 9 and 10). In  
153 May 2015, all these groups were deployed in BST-brand long line baskets (BST oyster supplies,  
154 Australia) on a long line system. All baskets were placed at the same tidal height in 3 replicates  
155 of 100 oysters per basket.

156

#### 157 **Ploidy analysis**

158 In May 2015, 15 individuals from 6 different crosses (3 half sib families from cross 2 using  
159 non-mosaic parents and 3 half sib families from cross 7 using mosaic parents – Fig. 1) were  
160 incubated for 8h in seawater containing 0.005% colchicine. Live weight and length were

161 measured. For each individual, the ploidy was analyzed by two different techniques: FCM and  
162 cytogenetics, the latter following the air drying technique of Thiriot-Quéveux and Ayraud  
163 (1982). Gills were dissected in seawater, with a small portion (4 mm<sup>2</sup>) used for FCM and the  
164 remaining gill used for later cytogenetic analysis.

165 Some histograms resulting from the FCM analysis were further analyzed using ModFit LT  
166 (Verity House Software, Topsham, Maine) for curve fitting. DNA content relative to the diploid  
167 standard was determined and the CV of DNA content in the population of cells was recorded for  
168 each group.

169 For cytogenetic analysis, the gill was treated for 30 min in 0.9% sodium citrate and fixed in a  
170 freshly prepared absolute alcohol-acetic acid (3:1) with three changes at 20 min. intervals. Slides  
171 were made from one individual gill following the air drying technique of Thiriot-Quéveux and  
172 Ayraud (1982). The preparations were stained for 20 min with Giemsa (4%, pH 6.8).  
173 Chromosome counts were made directly by microscope observation (Nikon Eclipse 50i with  
174 camera image acquisition incorporated Nikon DS-Fi1) of apparently intact metaphases. Thirty  
175 metaphases is the minimal statistical number per individual typically accepted in cytogenetic  
176 studies (Leitão et al. 2001b). The level of aneuploidy was estimated by counting the total  
177 number of aneuploid metaphases out of the total number of metaphases counted per individual.  
178 Counting of chromosomes of all the individuals was performed by the same observer (JDS) to  
179 eliminate subjectivity associated with different observers.

180

## 181 **Data analysis**

182 Statistical analyses were computed using STATGRAPHICS Centurion XV.II. Differences in  
183 chromosome counts between groups were assessed using the nonparametric Kruskal–Wallis test,

184 since the assumptions of normality were not met. Differences in length among groups during the  
185 larval stage were assessed using a one-way ANOVA at  $\alpha= 0.05$  and a Tukey's honestly  
186 significant difference (HSD) procedure, assuming equal variances. Because the assumptions of  
187 normality were not met for length and live weight of some groups as juveniles, the  
188 nonparametric Kruskal–Wallis test and a Dunn's procedure were performed. Differences in the  
189 relative DNA content at both larval and juvenile stages were assessed using a one-way ANOVA  
190 at  $\alpha= 0.05$  and a Tukey's HSD procedure, assuming equal variances.

191

192

## Results

### 193 Offspring performance

#### 194 Larvae

195 Larval survival was estimated from day 2 to 12 in all 30 families. No significant differences  
196 were found among all the triploid and tetraploid groups ( $p= 0.41$ ). As far as we know, this is the  
197 first paper comparing larval survival of three different ploidy groups in *C. virginica*. The lack of  
198 differences among the groups might be attributable to high variance among families within  
199 groups, especially 3F and 4N larvae. The source of the egg seemed a determinant in the survival  
200 of larvae. There was a positive correlation ( $R=0.58$ ,  $p=0.02$ ) in survival of tetraploid larvae and  
201 triploid larvae (4N vs 3F) made with the same eggs. There was no such correlation between  
202 tetraploid larvae and triploid larvae made from tetraploid sperm (4N vs 3M –  $R=-0.18$ ,  $p=0.001$ ).

203 We did not attempt to analyze larval growth rate among the families, but we measured the  
204 terminal size of oyster larvae just before setting. Significant differences were found among the  
205 groups ( $p<0.05$ ). Both spawns using mosaic and non-mosaic tetraploids as females (3F) had  
206 larger eyed larvae ( $342.6 \mu\text{m} \pm 15.4$ ,  $n= 3$  families and  $342.3 \mu\text{m} \pm 6.7$ ,  $n= 7$  families,

207 respectively) than other spawns but, was only significantly different from the triploid cross made  
208 from the male tetraploid non-mosaic ( $309.9 \mu\text{m} \pm 15.7$ ,  $n=6$  families) (Table 1). Otherwise,  
209 there was consistency in eyed larvae size according to cross, with 2n female x 4n male being the  
210 smallest (3M –  $310.1 \mu\text{m}$ ), 4n x 4n intermediate (4N –  $328.8 \mu\text{m}$ ), and 4n female x 2n male the  
211 largest (3F –  $342.4 \mu\text{m}$ ) (Table 1).

212

### 213 Juveniles

214 After 16 months, only 4 of 10 4n female x 2n male (3F) families and 8 of 10 4N families  
215 were still alive; all of the 2n female x 4n male (3M) families survived to 16 months. For juvenile  
216 survival overall, 3M families had the highest survival ( $94 \pm 4.9\%$  for non-mosaic and  $89 \pm 2.5\%$   
217 for mosaic tetraploid parents). Survival of 3F juvenile triploids (4n female x 2n male) had the  
218 lowest survival ( $21 \pm 2.1\%$  for non-mosaic and  $45 \pm 4.6\%$  for mosaic tetraploid parents).  
219 Tetraploids (4N) had intermediate survival (Fig. 3). Differences were significant among groups  
220 ( $p < 0.05$ ).

221 For juvenile length and live weight of families at 16 months, there was significant variation  
222 among the ploidy groups ( $p < 0.05$ ). The overall trend was that triploid groups were  
223 indistinguishable from each other but the tetraploids were smaller (Table 2, Fig. 4). Tetraploids  
224 made from mosaic parents were smaller than tetraploids made from non-mosaic parents and this  
225 difference was significant for live weight (Table 2).

226

### 227 **Relative DNA content**

#### 228 Spat

229 Ten spat from each cross were tested at 2 months old to verify ploidy before deployment to  
230 the field. For each sample we recorded a mean relative DNA content and the CV of the  
231 frequency distribution histogram that was generated by FCM. For all observations, at least  
232 10,000 cells were observed. For both mean relative DNA content and CV, none of the 20  
233 triploid families were different from one another ( $p= 0.301$ ,) nor were the 10 tetraploid families  
234 ( $p= 0.632$ ) (Table 3). For CV, no significant differences were found among any of the 30 crosses  
235 ( $p= 0.873$ ). However, FCM of tetraploid spat revealed some unexpected findings. First, three  
236 triploids were found in two cultures from non-mosaic parents (two in 4N1 and one in 4N3).  
237 Second, one mosaic individual was found in three cultures (4N2, 4N4 and 4N10), being the  
238 earliest reversion we have ever recorded (Table 3).

239

#### 240 Juveniles

241 Ten juveniles were sampled from the 6 families used for cytogenetic analysis at one year old,  
242 also by FCM. All diploid x tetraploid individuals sampled were triploid. Contrary to the spat,  
243 the triploid juveniles showed a significant difference between groups ( $p= 0.016$ ). Families with a  
244 non-mosaic female parent (3F) having a lower relative DNA content (1.45,  $n = 10$ ) than families  
245 using a non-mosaic male parent (3M, 1.49,  $n = 10$ ) (Table 4).

246 For tetraploid families (4N) using non-mosaic parents, only one individual out of 10 (10%)  
247 was mosaic, which is, having both triploid and tetraploid cell populations. However, in the  
248 tetraploid families using mosaic parents, four out of 10 (40%) had triploid and tetraploid cell  
249 populations. For the families using non-mosaic parents, average relative DNA content was 1.88  
250 ( $n = 9$ ) and average CV of 4.63 ( $n = 9$ ) for tetraploid cell populations. For the triploid cell  
251 population, the relative DNA content was 1.44 ( $n = 1$ ) and CV of 4.74 ( $n = 1$ ). One of the 10

252 individuals from this group was a triploid and was removed from the experiment. For the cross  
253 using mosaic parents, average relative DNA content was 1.99 ( $n = 10$ ) and average CV of 4.59 ( $n$   
254 = 10) for tetraploid cell populations. For the triploid cell populations, average relative DNA  
255 content was 1.54 ( $n = 4$ ) and average CV of 5.34 ( $n = 4$ ) (Table 4). On average, the ratio of the  
256 mean relative DNA content of the triploid cell population to the mean relative DNA content of  
257 the tetraploid population was 0.77 ( $n = 5$ ) slightly higher than the expected 0.75 (Table 4).

258

### 259 **Cytogenetic analysis**

260 Chromosome counts of triploid (4 families, 10 individuals per family, 30 counts per  
261 individual:  $n = 1200$ ) and tetraploid (2 families, 10 individuals per family, 30 counts per  
262 individual:  $n = 600$ ) juveniles were compiled (Fig. 5). For triploid juveniles, cells from progeny  
263 of non-mosaic and mosaic, males and females displayed a wide variation of chromosome  
264 number, ranging from 14 to 30 chromosomes (Figs. 5a, 5b, 5e, 5f). Interestingly, the number of  
265 metaphases showing 24, 26 and 28 chromosomes in triploid progenies are much higher than  
266 those showing 25, 27 or 29; a similar situation, although less marked, is also present in tetraploid  
267 progenies, with metaphases showing 34, 36 and 38 being higher than those showing 35, 37 or 39  
268 (Figs. 5a, 5b, 5e, 5f). Despite this wide variation, the mode of all triploid families ranged from  
269 28 to 30 chromosomes, among the 10 individuals (Table 5). More than 3/4 of all metaphase  
270 spreads from triploid embryos were aneuploid regardless of the origin of the tetraploid parent,  
271 non-mosaic vs mosaic or male vs female. For the families using a tetraploid male, 79% of  
272 chromosome spreads from the non-mosaic parent were aneuploidy and 76% from the mosaic  
273 parent (Table 5). For the families using a tetraploid female, 78% of cells from both the non-  
274 mosaic and mosaic tetraploid parents were aneuploid (Table 5).

275 There were no significant differences among the medians of the chromosome counts between  
276 triploid progeny from non-mosaic and mosaic tetraploid parents ( $p = 0.196$ ), between progeny  
277 from non-mosaic males and females ( $p = 0.853$ ), or between progeny from mosaic males and  
278 females ( $p = 0.825$ ).

279 Tetraploid juveniles produced from non-mosaic tetraploids or from mosaic tetraploids were  
280 also examined. Cells from either origin also had a wide variation of chromosome number,  
281 ranging from 17 to 40 chromosomes (Figs. 5c, 5d). Despite this wide variation, the mode was  
282 consistent. The tetraploid families using non-mosaic parents had a range of modes from 38 to 40  
283 chromosomes among the 10 individuals, whereas the tetraploid families using mosaic parents  
284 had a much wider range of modes, from 32 to 40 chromosomes (Table 5). As with triploids,  
285 about 3/4 of all metaphase spreads from tetraploid embryos were aneuploid, with 72% in  
286 progeny from non-mosaic parents and 80% for the cross using mosaic parents (Table 5). There  
287 was a statistically significant difference among the medians of the chromosome counts between  
288 these two types of crosses ( $p = 2 \times 10^{-6}$ ).

289 To examine the evolution of chromosome loss over time in triploid progeny, we compared  
290 the results from our last study using the same type of crosses on 1-h-old and 6-h-old triploid  
291 embryos (de Sousa et al., 2016). In de Sousa et al (2016), 1-h-old and 6-h-old triploids were  
292 examined at two time periods of the same cohort. The data reported here for juveniles represent  
293 the same type of crosses but from new cohorts. At 1-h-old, aneuploidy was considerably lower  
294 (10% for non-mosaic females and 8% for mosaic females) than in 6-h-old embryos (68% for  
295 non-mosaic females, 67% for non-mosaic males, 64% for mosaic females and 69% for mosaic  
296 males). In 1-year-olds reported here, aneuploidy was higher still (78% for non-mosaic females,  
297 79% for non-mosaic males, 77% for mosaic females and 76% for mosaic males) (Fig. 6).

298 Unfortunately, at 1-h-old, only the embryos from female tetraploids showed adequate metaphase  
299 spreads to perform chromosome counts, perhaps owing to the physical nature of the eggs (de  
300 Sousa et al., 2016).

301

### 302 **Correlation between size and aneuploidy**

303 No correlations were observed between live weight and percentage of aneuploidy in the  
304 triploid progeny using male ( $p = 0.257$ ) or female ( $p = 0.592$ ) tetraploid parents (Figs. 7, 3M,  
305 3F). There was, however, a significant negative linear correlation between percent aneuploidy in  
306 an individual and its live weight for tetraploids overall ( $p = 0.006$ ,  $r^2 = 0.1255$ ) (Fig. 7, 4N).  
307 Interestingly, this negative correlation was entirely due to the negative correlation in mosaics.  
308 When non-mosaic and mosaic individuals were tested separately, only the mosaic individuals  
309 showed this negative correlation (non-mosaic:  $y = 0.005x + 12.0$ ,  $p = 0.11$ ; mosaic:  $y = -0.58x +$   
310  $7.51$ ,  $p = 0.09$ ).

311

### 312 **Concordance between Cytogenetics and FCM**

313 Parallel cytogenetic and FCM data were obtained for all 60 individuals among 6 families. By  
314 flow cytometry, none of the triploids was mosaic as evidenced by presence of a single DNA  
315 content peak at the expected triploid level. For chromosome counts, however, there were clearly  
316 counts that occurred in the diploid range (15-24). When we binned these counts and plotted the  
317 histograms, every triploid individual had some “diploid” cells present (data not shown), which  
318 were not observed by FCM.

319 Examples of FCM and cytogenetic (chromosome number) histograms are shown in Figure 8  
320 for tetraploids. For tetraploid progeny, there was also a general lack of concordance between



321 FCM and chromosome counts (Figs. 8a, 8b). Here we binned counts into three ranges of ploidy  
322 corresponding to diploid ( $\leq 24$ ), triploid (25-34), and tetraploid ( $\geq 35$ ). These values should  
323 correspond to relative DNA contents (as measured by fluorescence) of 50, 75, and 100. In all  
324 cases, however, we disregarded the diploid (50) peak. Diploid peaks could be the result of either  
325 reversion to the diploid stage or the presence of di-haploid sperm from the tetraploid. We argue  
326 that the diploid cells observed through FCM were di-haploid sperm cells, and therefore not part  
327 of the chromosome instability story, due to the high frequency of diploid cells and because every  
328 one of the occurrences of diploid cells corresponded to a male. Thus, we were more interested in  
329 correspondence between the tetraploid and triploid peaks for FCM and chromosome counts.  
330 There was little agreement between FCM results and the chromosome counts in virtually every  
331 individual (Figs. 8a, 8b).

332

333

## Discussion

334 Our results contribute to the developing body of knowledge about the heritability of  
335 chromosome instability in polyploid oysters. From previous studies in our laboratory, we found  
336 no evidence of heritability for chromosome instability in triploid embryos and juveniles of *C.*  
337 *virginica*, as measured through both FCM (Matt and Allen 2014) and chromosome counts (de  
338 Sousa et al. 2016). That is, triploids produced from tetraploids with obvious chromosome loss  
339 (mosaics) and those produced from “stable” tetraploids (non-mosaics) had the same degree of  
340 aneuploidy. A thorough study of this same condition in tetraploid crosses, using stable and  
341 unstable parents, has not been accomplished until now. We confirmed that in tetraploid  $\times$   
342 tetraploid crosses, however, chromosome instability in the parent does matter. Additionally, we  
343 confirmed the progressive loss of chromosomes over time by comparing aneuploidy at one year

344 old versus earlier life stages (de Sousa et al. 2016). Finally, we observed generally poor  
345 concordance in ploidy evaluation between FCM and cytogenetics.

346

### 347 **Offspring performance**

348 For larval survival, although no significant differences were found among all the crosses,  
349 triploids from the male tetraploid parent (3M – mosaics and non-mosaics combined) had higher  
350 survival (0.46) than triploid (3F) or tetraploid (4N) progeny from the female tetraploid parent  
351 (0.32 and 0.39, respectively). The results among triploid groups are similar to those obtained by  
352 Guo et al. (1996) and Matt and Allen (2014) with triploid *C. gigas* and *C. virginica* larvae,  
353 respectively. That is, triploids made from the eggs of tetraploids had generally lower larval  
354 survival than triploids from diploid eggs. For tetraploid larvae, only one other report is available  
355 for comparison (Guo et al. 1996) in *C. gigas*. In that report, the tetraploid crosses (n=3) had an  
356 average survival of 0.17 versus 0.39 in our study with *C. virginica*. Besides the obvious species  
357 difference, Guo et al. (1996) were using F<sub>1</sub> tetraploids, that is, had just been mated compared to  
358 ours that were >F<sub>10</sub>. Domestication is likely to have improved tetraploid performance over the  
359 generations. Tetraploid eggs are not used in the production of triploid for commercial purposes.  
360 This has as much to do with logistics as with survival of tetraploid eggs, simply because the  
361 fecundity of males is vastly greater than that of females. The problem with low survival of  
362 triploid larvae using tetraploid eggs reinforces this practice (Guo et al. 1996; Matt and Allen  
363 2014).

364 Size of larvae was only compared at their terminal size as eyed larvae, although we do have  
365 data for sizes as D-stage. Not surprisingly, larvae derived from tetraploid eggs were larger at D-  
366 stage than those from diploid eggs (average 90.6 µm vs 78.2 µm, respectively) owing to the

367 difference in egg size. That is, tetraploid eggs of *C. virginica* average about 70  $\mu\text{m}$  diameter and  
368 diploids – 50  $\mu\text{m}$ . For eyed larvae length, measured during harvests from day 22 to day 27, 3F  
369 triploids were significantly larger than 4N or 3M. While the difference in the size of eyed larvae  
370 would be expected between those starting as tetraploid eggs and those starting with diploid ones,  
371 it is harder to account for the difference in eyed larvae size between the 3F and 4N groups, and  
372 especially since 4N survival was higher. Both started with the same egg source. Besides Guo et  
373 al. (1996), there have been no other reports of size of tetraploid eyed larvae produced from 4n x  
374 4n mating. Despite the marginally higher survival of tetraploid larvae overall, compared to 3F  
375 larvae, the smaller size of eyed larvae in 4N may be related to genome instabilities or regulatory  
376 incompatibilities that often accompany autopolyploidy (Chen 2007; Comai 2005).

377       Offspring of the three major types of crosses, 3F, 4N, and 3M, were exposed to the same  
378 conditions during their deployment period. Of ten families deployed from each group, only 4 –  
379 3F and 6 – 4N survived while all 3M families survived. In contrast to larval survival, there  
380 appeared to be no clear correlation between survival of juveniles sharing the same female,  
381 indicating that more than maternal effect is contributing to adult survival. Although tetraploid  
382 families had better survival, they were smaller. Between the non-mosaic and mosaic tetraploid  
383 parents, those produced from non-mosaics were larger and heavier. Survival, length, and live  
384 weight results from juvenile tetraploids suggests that, contrary to the triploid progeny from this  
385 and the previous work by Matt and Allen (2014), the use of non-mosaic or mosaic tetraploid  
386 parents influences the performance of the tetraploid progeny. Previous studies have shown that  
387 aneuploidy can adversely affect fitness in bivalves, as for example, size. Linking aneuploidy of  
388 this phenomenon to size differences has been observed in diploid oysters (Leitão et al. 2001b;  
389 Thiriot-Quévieux et al. 1992; Zouros et al. 1996), although, unlike diploids, chromosome loss

390 from polyploids does not appear to be associated with small size (Guo and Allen 1994; Wang et  
391 al. 1999). In the present study, no correlation was observed between live weight and degree of  
392 aneuploidy of triploid progeny. On the other hand, there was a negative correlation between  
393 degree of aneuploidy and live weight for tetraploids that seemed entirely driven by tetraploids of  
394 mosaic parents, which were also smaller than tetraploids produced from non-mosaic parents.  
395 Nevertheless, this correlation was not as strong as in the studies of diploid oysters mentioned  
396 above. That the negative effects of aneuploidy seem to be smaller in polyploids than in diploids  
397 may be due to the fact that in diploids, chromosome loss has the effect of haploidization, where  
398 deleterious or lethal effects are expressed alleles that are no longer masked (Zouros et al. 1996).  
399 On the other hand, in polyploids, considerable chromosome loss might be tolerated, with small  
400 phenotypic effect, due to their extra copies of all genes (Comai 2005). In fact, the presence of a  
401 majority of aneuploidy cells in all the polyploidy oysters suggests that as long as there is some  
402 redundancy ( $>2$ ) in genes, almost any chromosome constitution is viable.

403

#### 404 **Heritability of chromosome instability between non-mosaic and mosaic parents**

405 Based on FCM of spat sampled at 2 months, there were no differences among the 20 triploid  
406 families or among the 10 tetraploid families in mean relative DNA content. However, we found  
407 3 triploids in a total of two tetraploid cultures. Because of the close proximity and simultaneity  
408 of these 30 spawns during larval rearing, we believe this is a result of contamination since a  
409 likely genetic explanation for triploids from tetraploid crosses eludes us. For example, if non-  
410 disjunction or other mechanism of producing haploid gametes from tetraploid parents were the  
411 cause, then we may have seen diploid progeny in either of the 3F or 3M families that shared  
412 tetraploid gametes with the 4N families in question. In addition, flow cytometry analysis of

413 broodstock revealed that sperm from mosaic tetraploids were consistently di-haploid and  
414 virtually indistinguishable from sperm of non-mosaics. On a separate note, three families of the  
415 2 month old spat had one mosaic individual (10%). In previous work with tetraploid families,  
416 Ritter and Allen (2015) found mosaics in 2 month old individuals from tetraploid families. In  
417 that study, the percent of mosaics among 11 families ranged from 7% to 70%, with all 11  
418 families affected. However, Ritter and Allen (2015) did not discriminate among non-mosaic and  
419 mosaic crosses. In 1 year-old juveniles, we only evaluated two tetraploid families by FCM – the  
420 ones used for cytogenetic analysis. One of ten individuals (10%) of the progeny using non-  
421 mosaic parents was mosaic (with triploid and tetraploid cell populations), whereas four of ten  
422 (40%) were mosaic when mosaic tetraploids were the parents. Again, the only other comparison  
423 of rates of mosaicism in tetraploid families comes from Ritter and Allen (2015) where all 11  
424 tetraploid families studied had rates of mosaicism between 39% and 87%. The difference  
425 between these two studies highlights the variability among tetraploid crosses for just about every  
426 trait. Indeed, previous studies have suggested that aneuploidy might be influenced by genetic  
427 background, not only in diploids (Leitão et al. 2001a) but also in tetraploids (McCombie et al.  
428 2005) of *C. gigas*.

429 Leitão et al. (2001a) hypothesized a maternal effect in the inheritance of aneuploidy in  
430 diploid populations. They examined crosses made by two female parents that differed in their  
431 level of aneuploidy and observed that levels of aneuploidy in the female parents were positively  
432 correlated to levels of aneuploidy in progeny. The confirmation of this hypothesis could also be  
433 an important study for tetraploids, where perhaps it might be advantageous to eliminate mosaic  
434 females only.

435 Evolution of chromosome loss over time in triploid progeny was also examined by including  
436 data published in 1-h-old and 6-h-old embryos (de Sousa et al. 2016) with this study, with 1  
437 year-olds. At 1h post-fertilization (PF), when the embryos have 2-4 cells, aneuploidy was low,  
438 and increased greatly by 6h PF. Aneuploidy was higher still at 1 year, but only marginally  
439 compared to 6-h-old embryos. Thus, it seems clear that chromosome instability starts during  
440 early development. We have recently concluded studies of meiotic or early mitotic irregularities  
441 in polyploid *C. virginica* through immunostaining and confocal microscopy that seem to confirm  
442 that chromosome instability is an inherent feature of polyploid shellfish, as suggested by our  
443 hypothesis in de Sousa et al. (2016).

444 Although reversion was originally documented in triploids (Allen et al. 1996) and later  
445 confirmed in other studies (Erskine 2003; Hand et al. 1999; Zhou 2002), reversion is more of a  
446 problem in tetraploids than it is in triploids. None of the triploid individuals examined in this  
447 study were mosaic by FCM. On the other hand, chromosome counts revealed far more  
448 variability: chromosome numbers for triploids ranged from 16 to 38 in 6-h-old embryos (de  
449 Sousa et al. 2016) and 14 to 30 in juveniles. Despite this wide range, the mode for all triploid  
450 individuals was 30 chromosomes in embryos and 28 in juveniles. It is likely that the same  
451 triploid animals analyzed by FCM actually possess aneuploid cells and/or heteroploid mosaic  
452 cells, not detected by FCM.

453 Generally, in both triploid and tetraploid progeny, the number of aneuploid metaphases  
454 showing even chromosome numbers were higher than those showing odd chromosome numbers.  
455 This was a very peculiar finding. As far as we know, no other studies about aneuploidy in  
456 polyploids or diploids observed this same pattern. Since we assume aneuploidy progresses

457 through random mitotic events that have nothing to do with chromosome pairing, it is difficult to  
458 account for the loss of pairs of chromosomes that might explain this pattern.

459

#### 460 **Concordance between Cytogenetics and FCM**

461 In all families examined cytogenetically (3n: n=4; 4n: n=2), individuals were also analyzed  
462 by FCM. Although FCM allows rapid analysis of large numbers of cells, cytogenetics can detect  
463 smaller differences in DNA content through chromosome loss or gain. The majority of the  
464 studies comparing these two techniques were made in human cancers (e.g., Adeyinka et al. 2003;  
465 Al-Mozain et al. 2015; Shackney et al. 1990), with a wide range of concordance depending on  
466 the tumor type: 37%–73% for bladder cancer, 43% for prostate cancer, 30% for various solid  
467 tumors, 69% for pediatric solid tumors, 54% for bone and soft tissue tumors, and 54% for breast  
468 carcinomas (Adeyinka et al. 2003). Only a few similar studies have been made in bivalves and  
469 these concerned neoplasias, another form of cancer (Reno et al. 1994; Smolarz et al. 2005a,  
470 2005b). In bivalve neoplasias, the proportion of normal and affected individuals using flow  
471 cytometry was comparable to the proportion determined by chromosome analysis, using  
472 histology as the reference technique (Smolarz et al. 2005b, 2005a). However, we must make the  
473 distinction between the agreement between techniques for detection of abnormalities and the  
474 agreement (or not) between techniques for showing the same degree of abnormality. The above  
475 studies showed the former, but not the latter. In cells affected by neoplasia, chromosome  
476 numbers tend to be tetraploid and higher. Moreover, clams with neoplasia have a high mitotic  
477 index (up to 500 mitoses in gills of affected clams) compared to healthy clams (Smolarz et al.  
478 2005a). As far as we know, this study of ours is the first for assessing the ploidy in polyploid  
479 oysters using both techniques.

480 In our work, we observed a lack of concordance between the techniques. One possible  
481 reason for this lack of concordance could be the selective nature of sampling cells for  
482 cytogenetics. In our work, both FCM and cytogenetics used gill tissue. For obtaining  
483 chromosome spreads, only cells that are actively in the process of mitosis will become a  
484 chromosome spread. This is accomplished by arresting active mitotic divisions in metaphase  
485 with a spindle inhibitor (colchicine, here), when chromosomes are maximally condensed (and  
486 duplicated). Once arrested, chromosomes become detached from the metaphase plate and are  
487 more amenable to display when the nucleus ruptures during preparation. High mitotic index is  
488 essential for finding sufficient cells in the metaphase. FCM, on the other hand, is indiscriminate  
489 in the cells it targets. The DAPI stain formulation we used contains detergent to dissociate and  
490 lyse the cells with high magnesium concentrations to maintain the integrity of the nuclear  
491 membrane. The stain covalently bonds to DNA and fluorescence is emitted in accordance to  
492 DNA content of every cell that passes through the system. Cells in G1 phase are numerically the  
493 most predominant cell type, and, consequently, show up as the largest or even the unique peak in  
494 the FCM histograms (Rabinovitch 1994). For example, in a typical cycle of a human cell with a  
495 total cycle time of 24 hours, the G1 phase last about 11 hours, S phase about 8 hours, G2 about 4  
496 hours, and M only about 1 hour (Cooper 2000). In our experience, G2 peaks are very small and  
497 mostly absent in typical gill preparations for flow cytometry. G1 cells detected by FCM have  
498 half the DNA content of G2 and mitotic cells (cytogenetics). Consequently, the two techniques  
499 are analyzing cells in different cycle phases. One possibility for the discrepancy between  
500 cytogenetics and FCM may be the selectivity of sampling cells of these two techniques.

501 Mitotic index of oysters may contribute to the problem of lack of concordance. Because the  
502 mitotic index is usually low in marine invertebrate adults (Sole-Cava et al. 2013), chromosome



503 counts are mainly dependent on the animal condition. In our case, we tried to stimulate mitotic  
504 indices by sampling oysters that were growing in the spring time, when we know growth rate is  
505 high. Even so, mitotic spreads are rare in some cases, vastly outnumbered by interphase nuclei.  
506 In the present study, 30 cells per animal were analyzed, whereas, for example, in a study also  
507 comparing the two techniques in bivalves (Reno et al. 1994), 100 chromosome spreads per  
508 animal were considered. Therefore, it could be helpful in future studies to, whenever possible,  
509 increase the number of cells analyzed cytogenetically.

510 Another possible explanation for lack of concordance between FCM and chromosome counts  
511 is loss of chromosomes by artifact, for example during the preparation. The air drying technique  
512 of Thiriot-Quéveux and Ayraud (1982) is intended to promote chromosome spreading. During  
513 preparation it is possible that certain chromosomes of some metaphases are “over-spread” and/or  
514 overlapping, leading the observer to assume chromosomal loss and/or gain. To avoid this bias in  
515 this study, such apparent metaphases were avoided. In early cytogenetic work in human  
516 lymphocytes, a significant excess of hypoploid over hyperploid cells were often attributed to  
517 technical artifact (Ford et al. 1988). Indeed, in humans, due to the possible difficulty in  
518 distinguishing true aneuploidy from random loss, gain, or rearrangement as a result of technical  
519 artifact, guidelines have been established requiring, for example, the loss of the same  
520 chromosome in at least three cells, in order to become reportable (Arsham et al. 2017).  
521 Questionable gains or losses are always verified by checking the surrounded area to determine if,  
522 for example, a gain can be attributed to a neighboring metaphase (Arsham et al. 2017). In our  
523 case, since we are dealing with bivalves where the effects of somatic aneuploidy are tolerated,  
524 higher percentages of chromosome loss in polyploids seems logical and, therefore, judging  
525 artifact becomes more difficult. In other polyploid species, like autotetraploid yeast (Mayer and

526 Aguilera 1990) and polyploid plants (De Storme and Mason 2014), high levels of somatic  
527 aneuploidy also seem to be well tolerated. Somatic aneuploidy has been detected cytogenetically  
528 in several polyploid plants, e.g., *Arabidopsis suecica*, a natural allotetraploid (Wright et al.  
529 2009), potato-tomato hybrids (Wolters et al. 1994) and the Moscow salsify, *Tragopogon*  
530 *miscellus* (Chester et al. 2012). Higher genome redundancy in polyploid genomes can allow a  
531 greater tolerance of chromosome loss compared to diploid genomes (De Storme and Mason  
532 2014).

533 One final point deserves discussion. Previous work by Zhang et al. (2010) and Zhang et al.  
534 (2013) in triploid *C. gigas* and *C. ariakensis* led to a hypothesis for chromosome loss due to  
535 chromosome clumping during mitotic divisions. Mosaic individuals with more chromosome  
536 clumps in their cells tended to have higher percentages of aneuploidy. de Sousa et al. (2016)  
537 hypothesized that observations of clumping were consistent with chromosome loss due to  
538 supernumerary centrosomes, such that the formation of supernumerary centrosomes would  
539 encourage chromosome mis-segregation and leave partial karyotypes at large in the cell or in  
540 micro-cells. We cannot rule out that some of the chromosomes counts reported here were the  
541 product of counting a “clump.” As a result of the air drying technique, we can hypothesize that  
542 those clumps could dissociate from the original metaphase, giving the impression of a new  
543 highly aneuploid metaphase with enough scattered chromosomes to count. We think this is  
544 improbable but at the same time, a good number of peridiploid metaphases were found in  
545 tetraploids.

546

## 547 **Acknowledgements**

548 We would like to thank the entire staff of the Aquaculture Genetics and Breeding  
549 Technology Center (ABC) for their technical help. Special thanks to Shelley Katsuki for the  
550 larval rearing and Eric Guevelou for helpful discussion. We are grateful to an anonymous  
551 reviewer for pointing out the unusual patterns of chromosome loss favoring even numbered  
552 counts. This paper is Contribution No. xxxx of the Virginia Institute of Marine Science, College  
553 of William & Mary.

554

555

### References

556 Adeyinka A., Baldetorp B., Mertens F., Olsson H., Johannsson O., Heim S., Pandis N. 2003.  
557 Comparative cytogenetic and DNA flow cytometric analysis of 242 primary breast carcinomas.  
558 *Cancer Genet. Cytogenet.* 147, 62–67. doi:10.1016/S0165-4608(03)00190-0

559 Allen Jr. S.K., and Bushek D. 1992. Large-scale production of triploid oysters, *Crassostrea*  
560 *virginica* (Gmelin), using “stripped” gametes. *Aquaculture* 103, 241–251. doi:10.1016/0044-  
561 8486(92)90170-P

562 Allen Jr. S.K., Guo X., Burreson B., Mann R. 1996. Heteroploid mosaics and reversion  
563 among triploid oysters, *Crassostrea gigas*. Fact or artifact. *J Shellfish Res* 15, 514–522.

564 Allen S.K. 1988. Triploid oysters ensure year-round supply. *Oceanica*.

565 Allen S.K. 1983. Flow cytometry: Assaying experimental polyploid fish and shellfish.  
566 *Aquaculture* 33, 317–328. doi:10.1016/0044-8486(83)90412-X

567 Al-Mozain N., Mashi A., Belgaumi A., Al-Sweedan S., Rawas F., Ramadan S., et al. 2015.  
568 Discrepancies between DNA index by flow cytometry and cytogenetic studies in childhood ?-  
569 lymphoblastic leukemia. *J. Clin. Exp. Pathol.* doi:10.4172/2161-0681.S1.016

- 570 Arsham M.S., Barch M.J., Lawce H.J. 2017. The AGT Cytogenetics Laboratory Manual.  
571 John Wiley & Sons.
- 572 Chen Z.J. 2010. Molecular mechanisms of polyploidy and hybrid vigor. *Trends Plant Sci.* 15,  
573 57. doi:10.1016/j.tplants.2009.12.003
- 574 Chen Z.J. 2007. Genetic and epigenetic mechanisms for gene expression and phenotypic  
575 variation in plant polyploids. *Annu. Rev. Plant Biol.* 58, 377–406.  
576 doi:10.1146/annurev.arplant.58.032806.103835
- 577 Chester M., Gallagher J.P., Symonds V.V., Silva A.V.C., Mavrodiev E.V., Leitch A.R., et al.  
578 2012. Extensive chromosomal variation in a recently formed natural allopolyploid species,  
579 *Tragopogon miscellus* (Asteraceae). *Proc. Natl. Acad. Sci.* 109, 1176–1181.  
580 doi:10.1073/pnas.1112041109
- 581 Comai L. 2005. The advantages and disadvantages of being polyploid. *Nat. Rev. Genet.* 6,  
582 836–846. doi:10.1038/nrg1711
- 583 Cooper G.M. 2000. *The Eukaryotic Cell Cycle*.
- 584 Cornet M. 2000. Obtaining cell proliferation for chromosome preparation in gill tissue  
585 culture of the oyster *Crassostrea gigas*. *Cytotechnology* 32, 1–7. doi:10.1023/A:1008053305546
- 586 Cornet M. 1993. A short-term culture method for chromosome preparation from somatic  
587 tissues of adult mussel (*Mytilus edulis*). *Experientia* 49, 87–90. doi:10.1007/BF01928798
- 588 de Sousa J.T., Allen S.K., Baker H., Matt J.L., 2016. Aneuploid progeny of the American  
589 oyster, *Crassostrea virginica*, produced by tetraploid  $\times$  diploid crosses: another example of  
590 chromosome instability in polyploid oysters. *Genome* 59, 327–338. doi:10.1139/gen-2015-0222

- 591 de Sousa J.T., Matias D., Joaquim S., Ben-Hamadou R., Leitão A. 2011. Growth variation in  
592 bivalves: New insights into growth, physiology and somatic aneuploidy in the carpet shell  
593 *Ruditapes decussatus*. *J. Exp. Mar. Biol. Ecol.* 406, 46–53. doi:10.1016/j.jembe.2011.06.001
- 594 De Storme N., and Mason A. 2014. Plant speciation through chromosome instability and  
595 ploidy change: Cellular mechanisms, molecular factors and evolutionary relevance. *Curr. Plant*  
596 *Biol.* 1, 10–33. doi:10.1016/j.cpb.2014.09.002
- 597 Foighil D.O., and Thiriote-Quievreux C. 1991. Ploidy and Pronuclear Interaction in  
598 Northeastern Pacific *Lasaea* Clones (Mollusca: Bivalvia). *Biol. Bull.* 181, 222–231.
- 599 Ford J.H., Schultz C.J., Correll A.T. 1988. Chromosome elimination in micronuclei: a  
600 common cause of hypoploidy. *Am. J. Hum. Genet.* 43, 733–740.
- 601 Griffiths A.J., Gelbart W.M., Miller J.H., Lewontin R.C. 1999. Changes in Chromosome  
602 Number.
- 603 Guo X., and Allen Jr. S.K. 1994. Viable tetraploid Pacific oyster (*Crassostrea gigas*  
604 Thunberg) produced by inhibiting polar body I in eggs from triploids. *Mol. Mar. Biol.*  
605 *Biotechnol.* 3, 42–50.
- 606 Guo X., DeBrosse G.A., Allen Jr. S.K. 1996. All-triploid Pacific oysters (*Crassostrea gigas*  
607 Thunberg) produced by mating tetraploids and diploids. *Aquaculture* 142, 149–161.  
608 doi:10.1016/0044-8486(95)01243-5
- 609 Guo X., Wang Y., Xu Z., Yang H. 2009. Chromosome set manipulation in shellfish.  
610 *Woodhead Publ. Food Sci. Technol. Nutr.* 165–194.
- 611 Hand R.E., Nell J.A., Reid D.D., Smith I.R., Maguire G.B. 1999. Studies on triploid oysters  
612 in Australia: effect of initial size on growth of diploid and triploid Sydney rock oysters,

613 *Saccostrea commercialis* (Iredale & Roughley). *Aquac. Res.* 30, 34–42. doi:10.1046/j.1365-  
614 2109.1999.00296.x

615 Lee T. 1999. Polyploidy and Meiosis in the Freshwater Clam *Sphaerium striatinum*  
616 (Lamarck) and Chromosome Numbers in the Sphaeriidae (Bivalvia, Veneroidea). *Cytologia*  
617 (Tokyo) 64, 247–252. doi:10.1508/cytologia.64.247

618 Leitão A., Boudry P., McCombie H., Gérard A., Thiriot-Quévieux C. 2001a. Experimental  
619 evidence for a genetic basis to differences in aneuploidy in the Pacific oyster (*Crassostrea gigas*).  
620 *Aquat. Living Resour.* 14, 233–237. doi:10.1016/S0990-7440(01)01119-6

621 Leitão A., Boudry P., Thiriot-Quévieux C. 2001b. Negative correlation between aneuploidy  
622 and growth in the Pacific oyster, *Crassostrea gigas*: ten years of evidence. *Aquaculture* 193, 39–  
623 48. doi:10.1016/S0044-8486(00)00488-9

624 Matt J.L., and Allen S.K. 2014. Heteroploid mosaic tetraploids of *Crassostrea virginica*  
625 produce normal triploid larvae and juveniles as revealed by flow cytometry. *Aquaculture* 432,  
626 336–345. doi:10.1016/j.aquaculture.2014.05.015

627 Mayer V.W., and Aguilera A. 1990. High levels of chromosome instability in polyploids of  
628 *Saccharomyces cerevisiae*. *Mutat. Res. Mol. Mech. Mutagen.* 231, 177–186. doi:10.1016/0027-  
629 5107(90)90024-X

630 McCombie H., Lapègue S., Cornette F., Ledu C., Boudry P. 2005. Chromosome loss in bi-  
631 parental progenies of tetraploid Pacific oyster *Crassostrea gigas*. *Aquaculture, Genetics In*  
632 *Aquaculture VIII* Eighth International Symposium on Genetics in Aquaculture 247, 97–105.  
633 doi:10.1016/j.aquaculture.2005.02.003

- 634 Nell J.A. 2002. Farming triploid oysters. *Aquaculture* 210, 69–88. doi:10.1016/S0044-  
635 8486(01)00861-4
- 636 Park G.M., Yong T.S., Im K.I., Chung E.Y. 2000. Karyotypes of three species of *Corbicula*  
637 (*Bivalvia*: *Veneroida*) in Korea [WWW Document]. *J. Shellfish Res.* URL  
638 <https://eurekamag.com/research/010/897/010897899.php> (accessed 12.20.16).
- 639 Petkevičiūtė R., Stanevičiūtė G., Stunžėnas V., Lee T., Foighil D.Ó. 2007. Pronounced  
640 karyological divergence of the North American congeners *Sphaerium rhomboideum* and *S.*  
641 *occidentale* (*Bivalvia*: *Veneroida*: *Sphaeriidae*). *J. Molluscan Stud.* 73, 315–321.  
642 doi:10.1093/mollus/eym025
- 643 Piferrer F., Beaumont A., Falguière J.C., Flajšhans M., Haffray P., Colombo, L. 2009.  
644 Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance  
645 improvement and genetic containment. *Aquaculture* 293, 125–156.  
646 doi:10.1016/j.aquaculture.2009.04.036
- 647 Rabinovitch P.S. 1994. Chapter 18 DNA Content Histogram and Cell-Cycle Analysis.  
648 *Methods Cell Biol.*, *Flow Cytometry Second Edition*, Part A 41, 263–296. doi:10.1016/S0091-  
649 679X(08)61723-9
- 650 Reno P.W., House M., Illingworth A. 1994. Flow cytometric and chromosome analysis of  
651 softshell clams, *Mya arenaria*, with disseminated neoplasia. *J. Invertebr. Pathol.* 64, 163–172.  
652 doi:10.1016/S0022-2011(94)90025-6
- 653 Rinkevich B. 2005. Marine invertebrate cell cultures: new millennium trends. *Mar.*  
654 *Biotechnol. N. Y.* N 7, 429–439. doi:10.1007/s10126-004-0108-y

- 655 Ritter K., and Allen Jr. SK. 2015. Pilot study of family-based breeding of tetraploid  
656 *Crassostrea virginica*. *J. Shellfish Res.* 34: 674-674.
- 657 Shackney S.E., Burholt D.R., Pollice A.A., Smith C.A., Pugh R.P., Hartsock R.J. 1990.  
658 Discrepancies between flow cytometric and cytogenetic studies in the detection of aneuploidy in  
659 human solid tumors. *Cytometry* 11, 94–104. doi:10.1002/cyto.990110111
- 660 Smolarz K., Renault T., Soletchnik P., Wolowicz M. 2005a. Neoplasia detection in *Macoma*  
661 *balthica* from the Gulf of Gdansk: comparison of flow cytometry, histology and chromosome  
662 analysis. *Dis. Aquat. Organ.* 65, 187–195. doi:10.3354/dao065187
- 663 Smolarz K., Renault T., Wolowicz M. 2005b. Histology, cytogenetics and cytofluorimetry in  
664 diagnosis of neoplasia in *Macoma balthica* (*Bivalvia*, L.) from the southern Baltic Sea.  
665 *Caryologia* 58, 212–219.
- 666 Sole-Cava A., Russo C.A.M., Thorpe J.P. 2013. *Marine Genetics*. Springer Science &  
667 Business Media.
- 668 Storchova Z., and Kuffer C. 2008. The consequences of tetraploidy and aneuploidy. *J. Cell*  
669 *Sci.* 121, 3859–3866. doi:10.1242/jcs.039537
- 670 Suja C.P., Sukumaran N., Dharmaraj S. 2007. Effect of culture media and tissue extracts in  
671 the mantle explant culture of abalone, *Haliotis varia* Linnaeus. *Aquaculture* 271, 516–522.  
672 doi:10.1016/j.aquaculture.2007.04.086
- 673 Thiriot-Quéveux C., and Ayraud N. 1982. Les caryotypes de quelques espèces de bivalves et  
674 de gastéropodes marins. *Mar. Biol.* 70, 165–172. doi:10.1007/BF00397681



- 675 Thiriot-Quévieux C., Pogson G.H., Zouros E. 1992. Genetics of growth rate variation in  
676 bivalves: aneuploidy and heterozygosity effects in a *Crassostrea gigas* family. *Genome* 35, 39–  
677 45. doi:10.1139/g92-007
- 678 Thiriot-Quévieux C., Soyer F., Bovée F. de, Albert P. 1988. Unusual chromosome  
679 complement in the brooding bivalve *Lasaea consanguinea*. *Genetica* 76, 143–151.  
680 doi:10.1007/BF00058813
- 681 Wang Z., Guo X., Allen Jr. S.K., Wang R. 1999. Aneuploid Pacific oyster (*Crassostrea gigas*  
682 Thunberg) as incidentals from triploid production. *Aquaculture* 173, 347–357.  
683 doi:10.1016/S0044-8486(98)00457-8
- 684 Wolters A.M., Schoenmakers H.C., Kamstra S., Eden J., Koornneef M., Jong J.H. 1994.  
685 Mitotic and meiotic irregularities in somatic hybrids of *Lycopersicon esculentum* and *Solanum*  
686 *tuberosum*. *Genome* 37, 726–735.
- 687 Wright K.M., Pires J.C., Madlung A. 2009. Mitotic instability in resynthesized and natural  
688 polyploids of the genus *Arabidopsis* (Brassicaceae). *Am. J. Bot.* 96, 1656–1664.  
689 doi:10.3732/ajb.0800270
- 690 Yoshino T.P., Bickham U., Bayne C.J. 2013. Molluscan cells in culture: primary cell cultures  
691 and cell lines. *Can. J. Zool.* 91. doi:10.1139/cjz-2012-0258
- 692 Zhang Q., Yu H., Howe A., Chandler W., Allen Jr. S.K. 2010. Cytogenetic mechanism for  
693 reversion of triploids to heteroploid mosaics in *Crassostrea gigas* (Thunberg) and *Crassostrea*  
694 *ariakensis*. *Aquac. Res.* 41, 1658–1667. doi:10.1111/j.1365-2109.2010.02541.x

695 Zhang Z., Wang X., Zhang Q., Allen Jr. S.K. 2013. Cytogenetic mechanism for the  
696 aneuploidy and mosaicism found in tetraploid Pacific oyster *Crassostrea gigas* (Thunberg). *J.*  
697 *Ocean Univ. China* 13, 125–131. doi:10.1007/s11802-014-2318-x

698 Zouros E., Thiriot-Quievreux C., Kotoulas G. 1996. The negative correlation between  
699 somatic aneuploidy and growth in the oyster *Crassostrea gigas* and implications for the effects of  
700 induced polyploidization. *Genet. Res.* 68, 109–116. doi:10.1017/S0016672300033991

701

702

703

704

705

706

707

708

709

710

711

712

713

714

Draft

715 **Tables**

716 Table 1. Length ( $\mu\text{m}$ ) of *C. virginica* pediveliger larvae just before setting for each cross for  
 717 triploid families using tetraploid female non-mosaic (4n NM) and mosaic (4n M) parents x  
 718 diploid (2n) to create 3F families; using male non-mosaic and mosaic tetraploid parents to create  
 719 3M families, and using mosaic and non-mosaic parents to create 4N families.

		Male			
		2n	4n NM	4n M	
Female	2n	--	309.9 $\mu\text{m} \pm 15.7$ n= 6	310.3 $\mu\text{m} \pm 11.8$ n= 4	← 3M
	4n NM	342.3 $\mu\text{m}^* \pm 6.7$ n= 7	328.5 $\mu\text{m} \pm 25.4$ n= 5	--	
	4n M	342.6 $\mu\text{m}^* \pm 15.4$ n= 3	--	329.2 $\mu\text{m} \pm 17.2$ n= 5	← 4N

↑  
3F

720

721

722

723

724

725

726

727

728

729 Table 2. Length (mm) and live weight (g) of *C. virginica* juveniles for triploid families using  
 730 tetraploid female non-mosaic (4n NM) and mosaic (4n M) parents x diploid (2n) to create 3F  
 731 families using male non-mosaic and mosaic tetraploid parents to create 3M families, and using  
 732 mosaic and non-mosaic parents to create 4N families.

		Variable	Male			
			2n	4n NM	4n M	
Female	2n	length	--	88.3 mm ± 3.8	84.5 mm ± 2.9	← 3M
		live weight	--	68.3 g ± 4.0	67.0 g ± 4.2	
	4n NM	length	82.0 mm ± 4.4	72.3 mm ± 2.8	--	
		live weight	63.0 g ± 4.9	44.6 g ± 2.7	--	
	4n M	length	84.4 mm ± 4.8	--	59.2 mm ± 3.3	← 4N
		live weight	73.5 g ± 5.6	--	29.3* g ± 3.3	

733

↑  
3F

734

735

736

737

738

739

740

741

742 Table 3. Flow cytometric analysis of 2 month old spat, showing the average of the relative  
 743 DNA content and the average of the coefficient of variation (CV) for ten spat of *C. virginica*  
 744 from each family for cell populations of somatic cells: tetraploid (4n) and triploid cells (3n).

		Mean Relative DNA Content			
		3n		4n	
		Av. mean	Av. CV	Av. mean	Av. CV
Non Mosaic	3M 1	1.44	7.49		
	3M 2	1.36	6.11		
	3M 3	1.40	6.61		
	3M 4	1.43	6.59		
	3M 5	1.50	5.40		
	3M 6	1.51	6.08		
	3F 1	1.43	6.76		
	3F 2	1.40	5.27		
	3F 3	1.42	6.27		
	3F 4	1.37	5.50		
	3F 5	1.36	7.26		
	3F 6	1.39	5.40		
Mosaic	4N 1	1.44 <sup>1</sup>	5.65	1.78	5.52
	4N 2	1.32 <sup>2</sup>		1.89	4.8
	4N 3	1.42 <sup>1</sup>	6.08	1.84	5.5
	4N 4	1.40 <sup>2</sup>	9.88	1.72	9.22
	4N 5			1.74	6.72
	3M 7	1.41	5.72		
	3M 8	1.45	5.32		
	3M 9	1.39	5.75		
	3M 10	1.39	6.24		
	3F 7	1.37	7.27		
3F 8	1.43	5.36			
3F 9	1.47	5.22			
3F 10	1.46	5.88			
4N 6			1.88	5.96	
4N 7			1.81	6.39	
4N 8			1.79	6.62	
4N 9			1.78	6.60	
4N 10	1.2 <sup>2</sup>	17.16	1.8	6.34	

745 <sup>1</sup> Pure triploids found in these cultures (two in 4N1 and one in 4N3).

746 <sup>2</sup> One mosaic individual found in each of these cultures (4N2, 4N4 and 4N10).

747 Table 4. Flow cytometric analysis of 1 year old juveniles, showing the average of the relative  
 748 DNA content and the average of the coefficient of variation (CV) for ten juveniles of *C.*  
 749 *virginica* from each family for cell populations of somatic cells: tetraploid (4n) and triploid cells  
 750 (3n). For mosaics, the ratio of triploid to tetraploid relative DNA content (3n/4n ratio) for each  
 751 cross is shown.

		Mean Relative DNA Content				3n/4n
		3n		4n		
		Av. mean	Av. CV	Av. mean	Av. CV	Ratio
Non Mosaic	3F	1.45	4.86			
	4N	1.44	4.74	1.88	4.63	0.77
	3M	1.49	5.15			
-----						
Mosaic	3F	1.46	5.22			
	4N	1.54	5.34	1.99	4.59	0.77
	3M	1.48	5.09			

752

753

754

755

756

757

758

759

760 Table 5. Chromosome count data and percentage of aneuploidy for ten juveniles of *C.*  
761 *virginica* from each family for triploid families using tetraploid female non-mosaic (4n NM) and  
762 mosaic (4n M) parents x diploid (2n) to create 3F families; using male non-mosaic and mosaic  
763 tetraploid parents to create 3M families, and using mosaic and non-mosaic parents to create 4N  
764 families. Numbers in boxes in each column indicate high and low values.

765

766

767

768

769

770

771

772

773

774

775

776

777

778

779

780

781

782

Draft

	Non-Mosaic			Mosaic		
	Mode	Average	% Aneuploidy	Mode	Average	% Aneuploidy
<b>3F</b>						
1	30	26.8	73	30	26.8	77
2	30	26.5	77	30	26.9	77
3	28	26.6	77	30	27.7	67
4	28	27.1	80	28	27.4	73
5	30	27.3	67	29	26.6	80
6	28	26.2	80	30	26.9	70
7	30	28.1	60	28	26.0	90
8	28	26.8	93	28	26.3	83
9	28	26.0	83	28	26.6	90
#	28	25.3	90	30	27.0	70
$\bar{x}$	29	26.7	78	29	26.8	78
<b>4N</b>						
1	40	34.4	73	38	32.2	77
2	40	35.1	73	40	35.4	73
3	38	37.1	80	38	33.1	77
4	38	35.0	80	30	30.6	80
5	39	33.5	83	40	33.8	67
6	38	32.3	77	38	32.6	73
7	–	–	–	32	30.3	90
8	40	37.0	70	36	33.5	93
9	40	36.6	57	28	33.5	90
#	40	37.1	53	40	36.3	77
$\bar{x}$	39	35.4	72	36	33.1	80
<b>3M</b>						
1	28	26.8	87	30	25.7	70
2	30	27.6	63	28	26.1	83
3	30	26.8	77	30	26.8	67
4	28	27.7	73	30	26.5	80
5	28	26.7	80	30	26.8	77
6	26	27.2	73	30	27.0	77
7	28	26.4	87	30	28.0	70
8	26	25.1	83	30	26.0	80
9	28	27.5	80	30	26.3	70
#	28	26.3	83	28	26.8	87
$\bar{x}$	28	26.8	79	30	26.6	76



**784 Figure captions**

785

786 Figure 1. *C. virginica* crossing design with a total of 30 families: 10 triploid families were  
787 produced using non-mosaic (7 families; no boxes) or mosaic tetraploid female parents (3  
788 families; in boxes) – 3F; 10 triploid families were produced using non-mosaic (6 families; no  
789 boxes) or mosaic tetraploid male parents (4 families; in boxes) – 3M; 10 tetraploid x tetraploid  
790 families were produced, 5 families between non-mosaic parents and 5 families between with one  
791 or more mosaic parents. For the diploid half of triploid crosses, a single male and a single  
792 female diploid were split 10 ways to produce 3M and 3F crosses, respectively. Crosses 2 and 7  
793 were used for subsequent cytogenetic analysis (arrows pointing right).

794 Figure 2. *C. virginica* larval survival estimated from day 2 to 12 in all 30 families.  
795 Horizontal bar represents overall average for non-mosaic (solid bars) and mosaic (checkered  
796 bars) families. The last solid bar in the 3M group corresponds to non-mosaic family 10 and its  
797 value was included in the overall average represented by horizontal bar over the values for the  
798 other non-mosaics families at the left.

799 Figure 3. *C. virginica* juveniles survival estimated at 1 year old the 30 families deployed to  
800 the field. Each group of crosses had 10 families. 0 denotes complete mortality in corresponding  
801 family. Horizontal bar represents overall average for non-mosaic (solid bars) and mosaic  
802 (checkered bars) families. The last solid bar in the 3M group corresponds to non-mosaic family  
803 10 and its value was included in the overall average represented by horizontal bar over the values  
804 for the other non-mosaics families at the left.

805

806 Figure 4. *C. virginica* juveniles live weight estimated at 1 year old in the 22 out of 30  
807 families that survived through winter. Horizontal bar represents overall average for non-mosaic  
808 (solid bars) and mosaic (checkered bars) families.

809 Figure 5. Compiled frequency distribution of chromosome number of cells from triploid *C.*  
810 *virginica* juveniles produced by crossing either tetraploid non-mosaic females (a) or mosaic  
811 females (b) with a diploid male (10 individuals per family), tetraploid non-mosaic males (e) or  
812 mosaic males (f) with a diploid female (10 individuals per family) and from tetraploid *C.*  
813 *virginica* juveniles produced by crossing non-mosaic tetraploid x non-mosaic tetraploid (c) or  
814 mosaic tetraploid x mosaic tetraploid (d) (10 individuals per family).

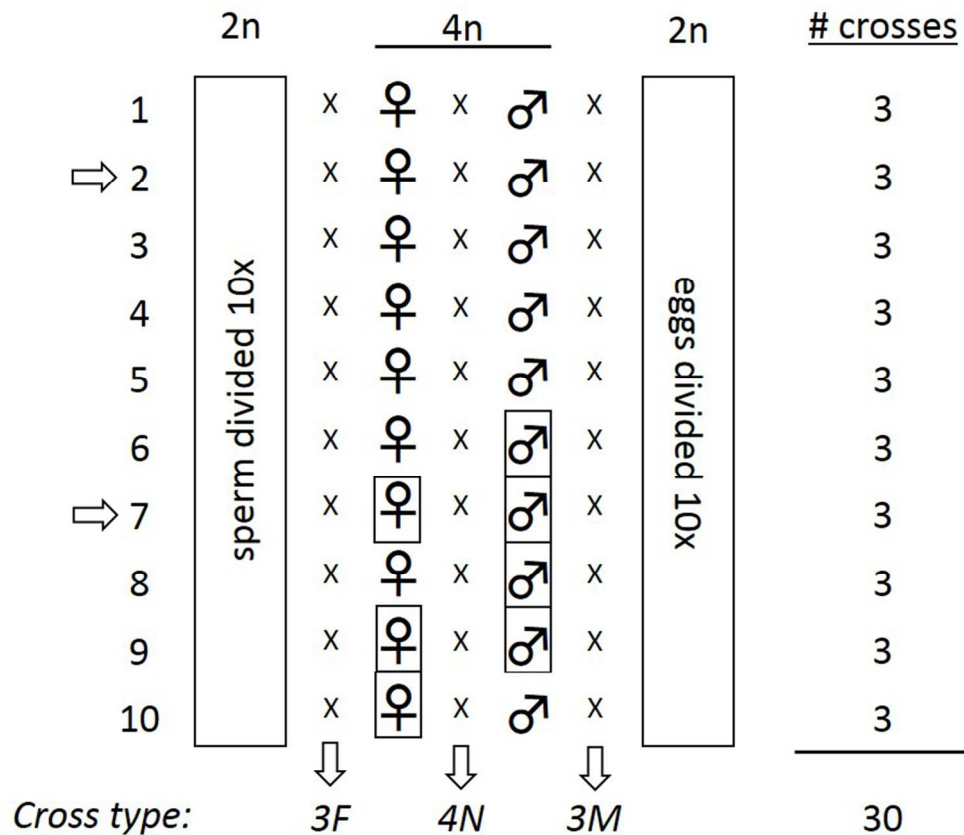
815 Figure 6. Percent aneuploidy in various crosses shows the evolution of chromosome loss in  
816 triploid progeny of *C. virginica* from 1-h-old embryos until 1-year-old juveniles produced by  
817 crossing either tetraploid non-mosaic female (NM female) or mosaic tetraploid female (M  
818 Female) with a diploid male or tetraploid non-mosaic male (NM Male) or mosaic male (M Male)  
819 with a diploid female. Comparison with the results from de Sousa et al. (2016) using the same  
820 type of crosses on 1-h-old and 6-h-old triploid embryos.

821 Figure 7. Correlation between live weight and percentage of aneuploidy in the triploid  
822 progeny of *C. virginica* produced by crossing either tetraploid non-mosaic or mosaic tetraploid  
823 females with a diploid male (3F), tetraploid non-mosaic or mosaic males with a diploid female  
824 (3M) and in the tetraploid progeny produced by crossing non-mosaic or mosaic tetraploids (4N).  
825 Solid circle – non-mosaic; open circle – mosaic.

826 Figure 8. Parallel cytogenetic and FCM data are shown for the tetraploid progeny, obtained  
827 by crossing non-mosaic x non-mosaic tetraploids (4N Non-Mosaic, a) or mosaic x mosaic

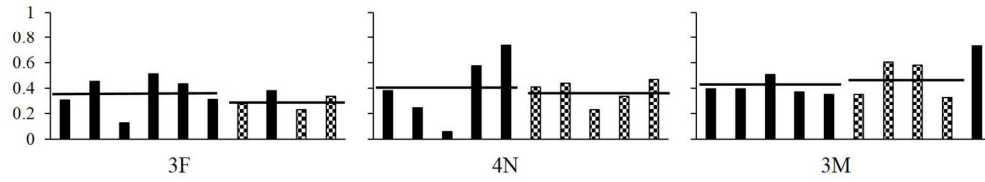
828 tetraploids (4N Mosaic, b). Chromosome counts were binned into three categories:  $\leq 24$  (first  
829 bar), 25-34 (second bar), and  $\geq 35$  (third bar) corresponding to 2n, 3n, and 4n ranges. These 3  
830 categories correspond to the 3 main peaks frequently observed in the FCM histograms.

Draft



*C. virginica* crossing design with a total of 30 families: 10 triploid families were produced using non-mosaic (7 families; no boxes) or mosaic tetraploid female parents (3 families; in boxes) – 3F; 10 triploid families were produced using non-mosaic (6 families; no boxes) or mosaic tetraploid male parents (4 families; in boxes) – 3M; 10 tetraploid x tetraploid families were produced, 5 families between non-mosaic parents and 5 families between with one or more mosaic parents. For the diploid half of triploid crosses, a single male and a single female diploid were split 10 ways to produce 3M and 3F crosses, respectively. Crosses 2 and 7 were used for subsequent cytogenetic analysis (arrows pointing right).

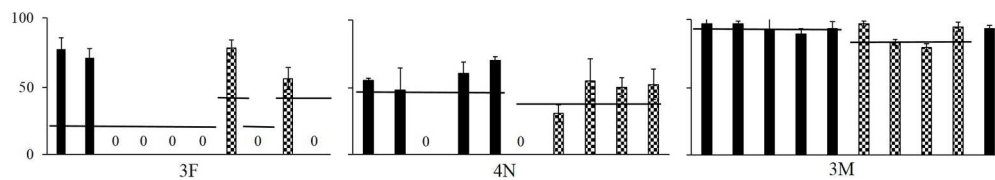
156x137mm (300 x 300 DPI)



*C. virginica* larval survival estimated from day 2 to 12 in all 30 families. Horizontal bar represents overall average for non-mosaic (solid bars) and mosaic (checkered bars) families. The last solid bar in the 3M group corresponds to non-mosaic family 10 and its value was included in the overall average represented by horizontal bar over the values for the other non-mosaics families at the left.

274x60mm (300 x 300 DPI)

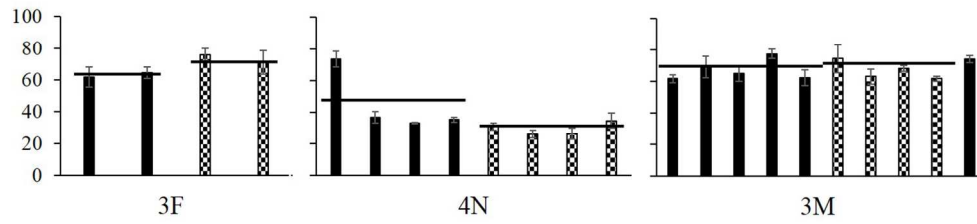
Draft



*C. virginica* juveniles survival estimated at 1 year old the 30 families deployed to the field. Each group of crosses had 10 families. 0 denotes complete mortality in corresponding family. Horizontal bar represents overall average for non-mosaic (solid bars) and mosaic (checkered bars) families. The last solid bar in the 3M group corresponds to non-mosaic family 10 and its value was included in the overall average represented by horizontal bar over the values for the other non-mosaics families at the left.

320x55mm (300 x 300 DPI)

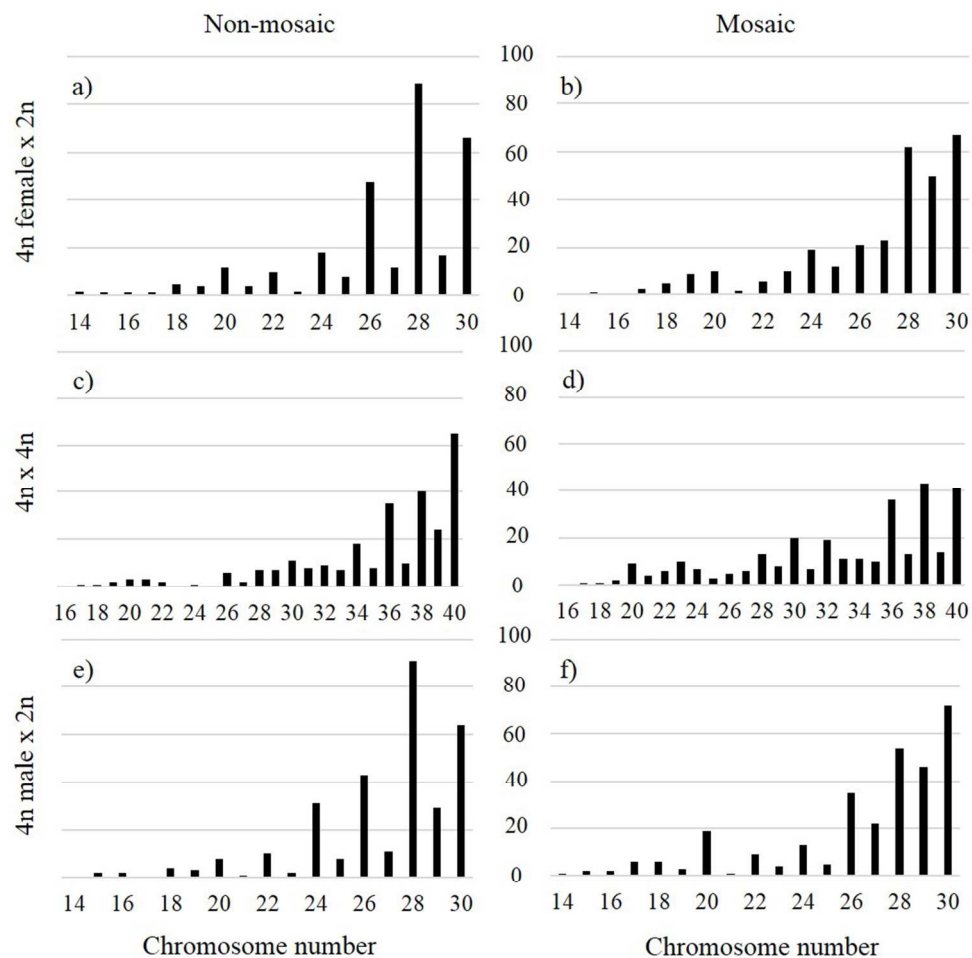
Draft



*C. virginica* juveniles live weight estimated at 1 year old in the 22 out of 30 families that survived through winter. Horizontal bar represents overall average for non-mosaic (solid bars) and mosaic (checkered bars) families.

236x62mm (300 x 300 DPI)

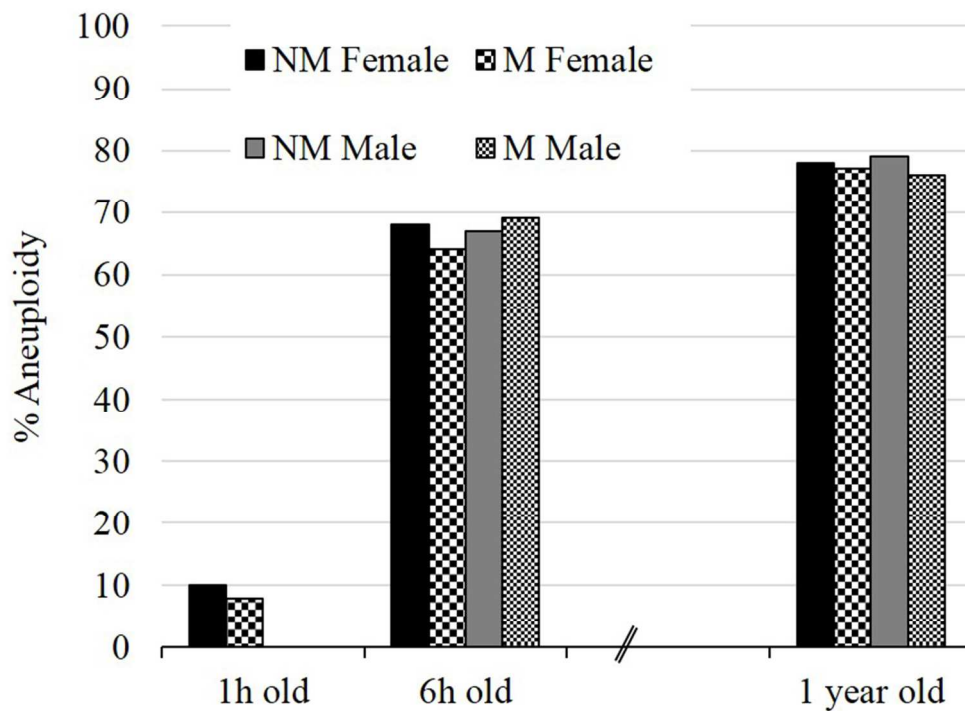
Draft



Compiled frequency distribution of chromosome number of cells from triploid *C. virginica* juveniles produced by crossing either tetraploid non-mosaic females (a) or mosaic females (b) with a diploid male (10 individuals per family), tetraploid non-mosaic males (e) or mosaic males (f) with a diploid female (10 individuals per family) and from tetraploid *C. virginica* juveniles produced by crossing non-mosaic tetraploid x non-mosaic tetraploid (c) or mosaic tetraploid x mosaic tetraploid (d) (10 individuals per family).

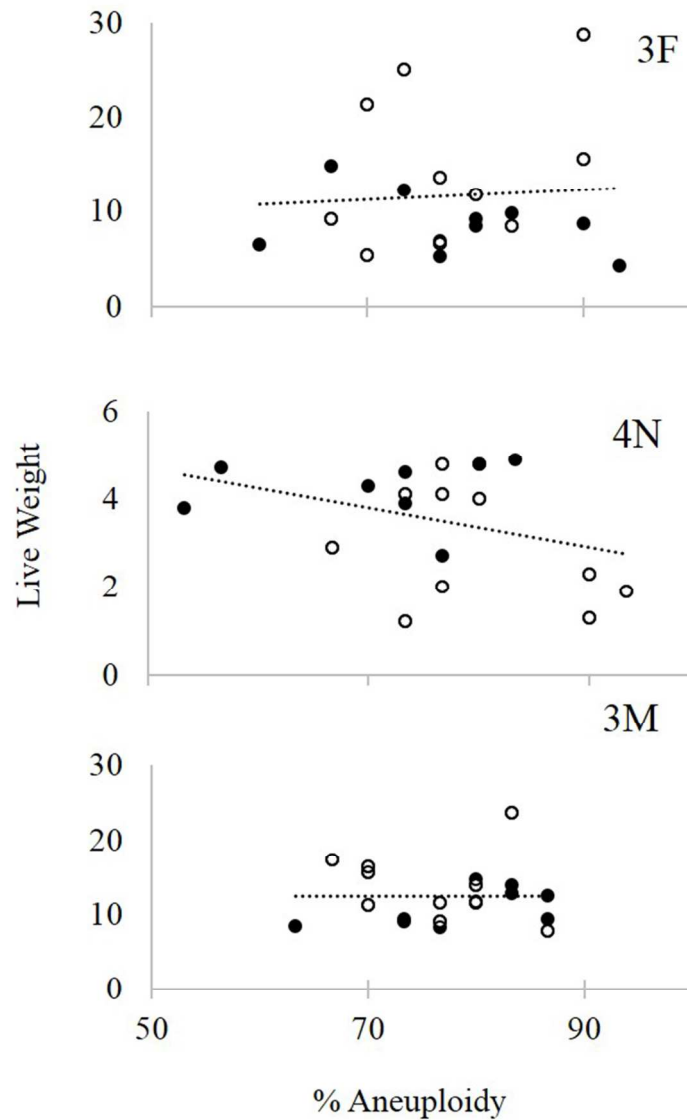
183x180mm (300 x 300 DPI)





Percent aneuploidy in various crosses shows the evolution of chromosome loss in triploid progeny of *C. virginica* from 1-h-old embryos until 1-year-old juveniles produced by crossing either tetraploid non-mosaic female (NM female) or mosaic tetraploid female (M Female) with a diploid male or tetraploid non-mosaic male (NM Male) or mosaic male (M Male) with a diploid female. Comparison with the results from de Sousa et al. (2016) using the same type of crosses on 1-h-old and 6-h-old triploid embryos.

152x111mm (300 x 300 DPI)



Correlation between live weight and percentage of aneuploidy in the triploid progeny of *C. virginica* produced by crossing either tetraploid non-mosaic or mosaic tetraploid females with a diploid male (3F), tetraploid non-mosaic or mosaic males with a diploid female (3M) and in the tetraploid progeny produced by crossing non-mosaic or mosaic tetraploids (4N). Solid circle – non-mosaic; open circle – mosaic.

109x175mm (300 x 300 DPI)

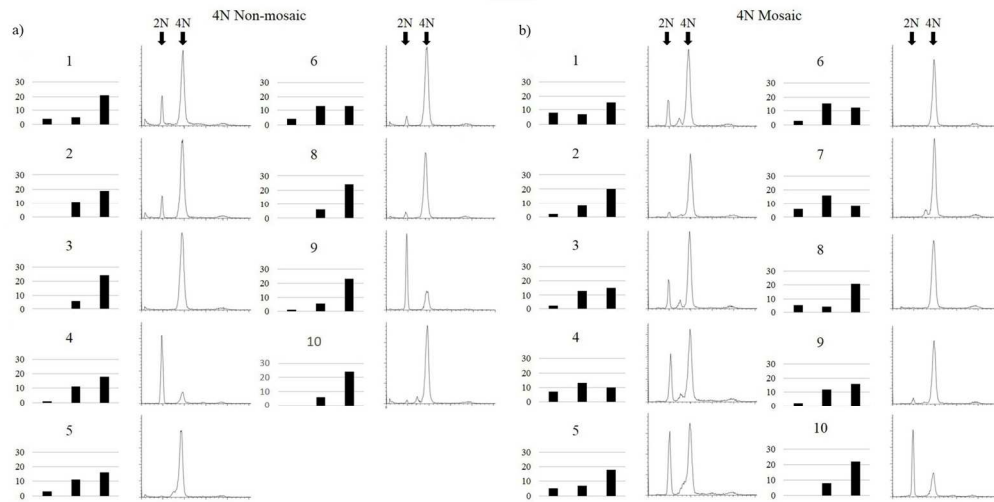


Figure 8. Parallel cytogenetic and FCM data are shown for the tetraploid progeny, obtained by crossing non-mosaic x non-mosaic tetraploids (4N Non-Mosaic, a) or mosaic x mosaic tetraploids (4N Mosaic, b). Chromosome counts were binned into three categories:  $\leq 24$  (first bar), 25-34 (second bar), and  $\geq 35$  (third bar) corresponding to 2n, 3n, and 4n ranges. These 3 categories correspond to the 3 main peaks frequently observed in the FCM histograms.

334x170mm (300 x 300 DPI)