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

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Abstract

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

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

Introduction

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

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

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

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

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

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

Materials and methods

Experimental population and crosses

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

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

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

Larval rearing

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

On day 2, in order to calculate the total number of larvae in the culture and their length, larvae were isolated on a top (48 µm) and bottom sieve (35 µm). Larvae collected on the 48 µm sieve on day 2 were returned to culture until day 4, at which time they were isolated on larger and larger sieve sizes until setting started on day 17, following standard protocol. Larvae were sampled on day 8 for ploidy analysis. Number of larvae collected on sieves was estimated during each water change by counting three aliquots at appropriate dilutions. An average size 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 μm).
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 °C to 30 °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

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

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

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

Data analysis

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

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

Results

Offspring performance

Larvae

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

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

respectively) than other spawns but, was only significantly different from the triploid cross made from the male tetraploid non-mosaic (309.9 µm ± 15.7, n= 6 families) (Table 1). Otherwise, there was consistency in eyed larvae size according to cross, with 2n female x 4n male being the smallest (3M – 310.1 µm), 4n x 4n intermediate (4N – 328.8 µm), and 4n female x 2n male the largest (3F – 342.4 µm) (Table 1).

Juveniles

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

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

Relative DNA content

Spat

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

Juveniles

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

For tetraploid families (4N) using non-mosaic parents, only one individual out of 10 (10%) was mosaic, which is, having both triploid and tetraploid cell populations. However, in the tetraploid families using mosaic parents, four out of 10 (40%) had triploid and tetraploid cell populations. For the families using non-mosaic parents, average relative DNA content was 1.88 (n = 9) and average CV of 4.63 (n = 9) for tetraploid cell populations. For the triploid cell 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).

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

Tetraploid juveniles produced from non-mosaic tetraploids or from mosaic tetraploids were also examined. Cells from either origin also had a wide variation of chromosome number, ranging from 17 to 40 chromosomes (Figs. 5c, 5d). Despite this wide variation, the mode was consistent. The tetraploid families using non-mosaic parents had a range of modes from 38 to 40 chromosomes among the 10 individuals, whereas the tetraploid families using mosaic parents had a much wider range of modes, from 32 to 40 chromosomes (Table 5). As with triploids, about 3/4 of all metaphase spreads from tetraploid embryos were aneuploid, with 72% in progeny from non-mosaic parents and 80% for the cross using mosaic parents (Table 5). There was a statistically significant difference among the medians of the chromosome counts between these two types of crosses ($p = 2x10^{-6}$).

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

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

Correlation between size and aneuploidy

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

Concordance between Cytogenetics and FCM

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

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

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

Discussion

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

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

Offspring performance

For larval survival, although no significant differences were found among all the crosses, triploids from the male tetraploid parent (3M – mosaics and non-mosaics combined) had higher survival (0.46) than triploid (3F) or tetraploid (4N) progeny from the female tetraploid parent (0.32 and 0.39, respectively). The results among triploid groups are similar to those obtained by Guo et al. (1996) and Matt and Allen (2014) with triploid *C. gigas* and *C. virginica* larvae, respectively. That is, triploids made from the eggs of tetraploids had generally lower larval survival than triploids from diploid eggs. For tetraploid larvae, only one other report is available for comparison (Guo et al. 1996) in *C. gigas*. In that report, the tetraploid crosses (n=3) had an average survival of 0.17 versus 0.39 in our study with *C. virginica*. Besides the obvious species difference, Guo et al. (1996) were using F_1 tetraploids, that is, had just been mated compared to ours that were $>F_{10}$. Domestication is likely to have improved tetraploid performance over the generations. Tetraploid eggs are not used in the production of triploid for commercial purposes. This has as much to do with logistics as with survival of tetraploid eggs, simply because the fecundity of males is vastly greater than that of females. The problem with low survival of triploid larvae using tetraploid eggs reinforces this practice (Guo et al. 1996; Matt and Allen 2014).

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

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

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

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

Heritability of chromosome instability between non-mosaic and mosaic parents

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

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

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

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

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

Generally, in both triploid and tetraploid progeny, the number of aneuploid metaphases showing even chromosome numbers were higher than those showing odd chromosome numbers. This was a very peculiar finding. As far as we know, no other studies about aneuploidy in 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.

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

Mitotic index of oysters may contribute to the problem of lack of concordance. Because the 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-Quiéveux 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

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

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

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Tables

Table 1. Length (µm) of *C. virginica* pediveliger larvae just before setting for each cross for triploid families using tetraploid female non-mosaic (4n NM) and mosaic (4n M) parents x diploid (2n) to create 3F families; using male non-mosaic and mosaic tetraploid parents to create 3M families, and using mosaic and non-mosaic parents to create 4N families.

		Male		
		2n	4n NM	4n M
Female	2n	--	309.9 µm ± 15.7 n= 6	310.3 µm ± 11.8 n= 4 ← 3M
	4n NM	342.3 µm* ± 6.7 n= 7	328.5 µm ± 25.4 n= 5	--
	4n M	342.6 µm* ± 15.4 n= 3	--	329.2 µm ± 17.2 n= 5 ← 4N

↑ 3F

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.

			Male		
		Variable	2n	4n NM	4n M
Female	2n	length	--	88.3 mm ± 3.8	84.5 mm ± 2.9
		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
		live weight	73.5 g ± 5.6	--	29.3* g ± 3.3

3M

4N

3F

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Table 3. Flow cytometric analysis of 2 month old spat, showing the average of the relative DNA content and the average of the coefficient of variation (CV) for ten spat of *C. virginica* 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		
	4N 1	1.44[1]	5.65	1.78	5.52
	4N 2	1.32[2]		1.89	4.8
	4N 3	1.42[1]	6.08	1.84	5.5
	4N 4	1.40[2]	9.88	1.72	9.22
	4N 5			1.74	6.72
Mosaic	**3M 7**	1.41	5.72		
	3M 8	1.45	5.32		
	3M 9	1.39	5.75		
	3M 10	1.39	6.24		
	3F7	1.37	7.27		
	3F8	1.43	5.36		
	3F9	1.47	5.22		
	3F10	1.46	5.88		
	4N6			1.88	5.96
	4N7			1.81	6.39
	4N8			1.79	6.62
	4N9			1.78	6.60
	4N10	1.2[2]	17.16	1.8	6.34

[1] Pure triploids found in these cultures (two in 4N1 and one in 4N3).

[2] 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			

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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.
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	Non-Mosaic			Mosaic		
	Mode	**Average**	**% Aneuploidy**	**Mode**	**Average**	**% Aneuploidy**
			3F			
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
x̄	**29**	**26.7**	**78**	**29**	**26.8**	**78**
			4N			
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
x̄	**39**	**35.4**	**72**	**36**	**33.1**	**80**
			3M			
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
x̄	**28**	**26.8**	**79**	**30**	**26.6**	**76**

783

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.

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Figure 4. *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.

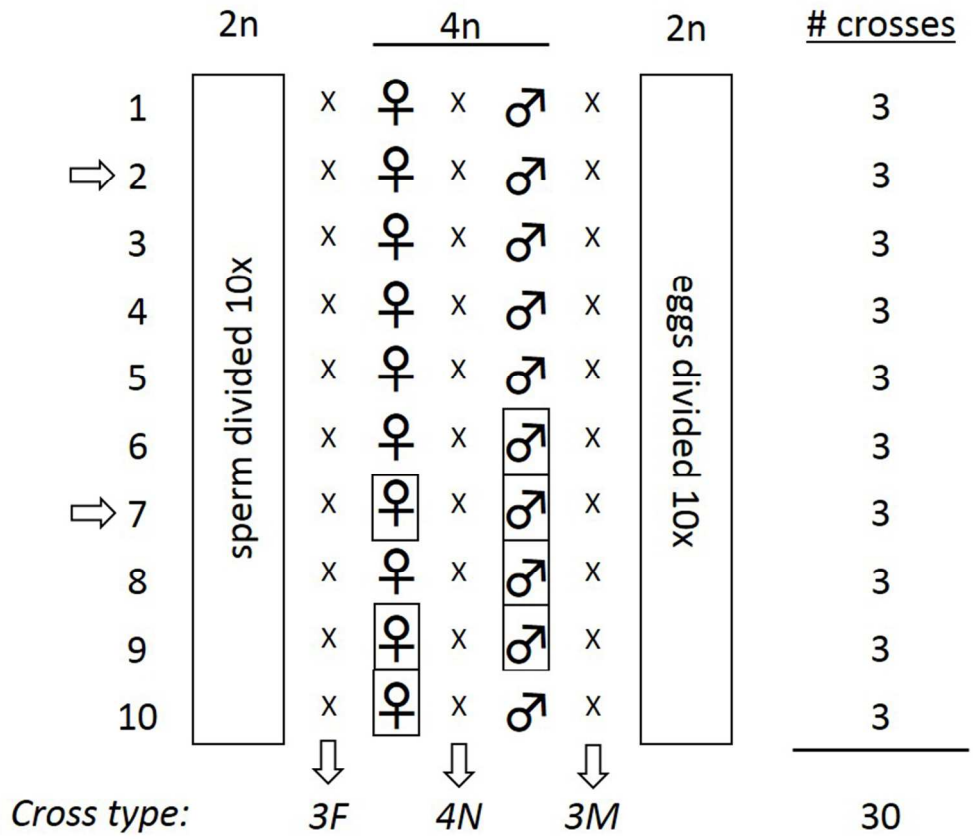
Figure 5. Compiled frequency distribution of chromosome number of cells from triploid *C. virginica* juveniles produced by crossing either tetraploid non-mosaic females (a) or mosaic females (b) with a diploid male (10 individuals per family), tetraploid non-mosaic males (e) or mosaic males (f) with 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).

Figure 6. 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.

Figure 7. 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.

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

828 tetraploids (4N Mosaic, b). Chromosome counts were binned into three categories: ≤24 (first
829 bar), 25-34 (second bar), and ≥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.



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).

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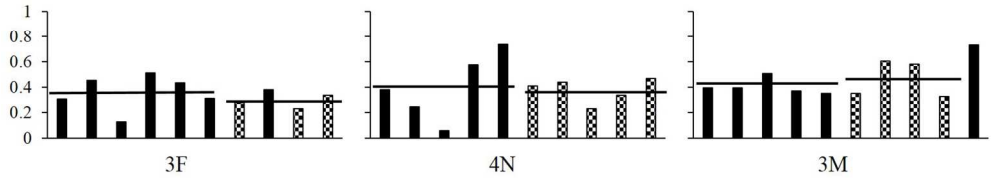
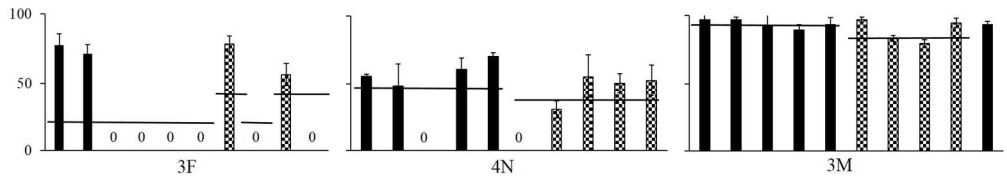


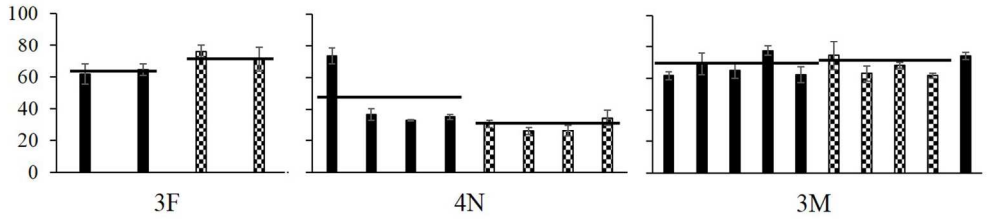
C. virginica larval survival estimated from day 2 to 12 in all 30 families. Horizontal bar represents overall average for non-mosaic (solid bars) and mosaic (checkered bars) families. The last solid bar in the 3M group corresponds to non-mosaic family 10 and its value was included in the overall average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

274x60mm (300 x 300 DPI)



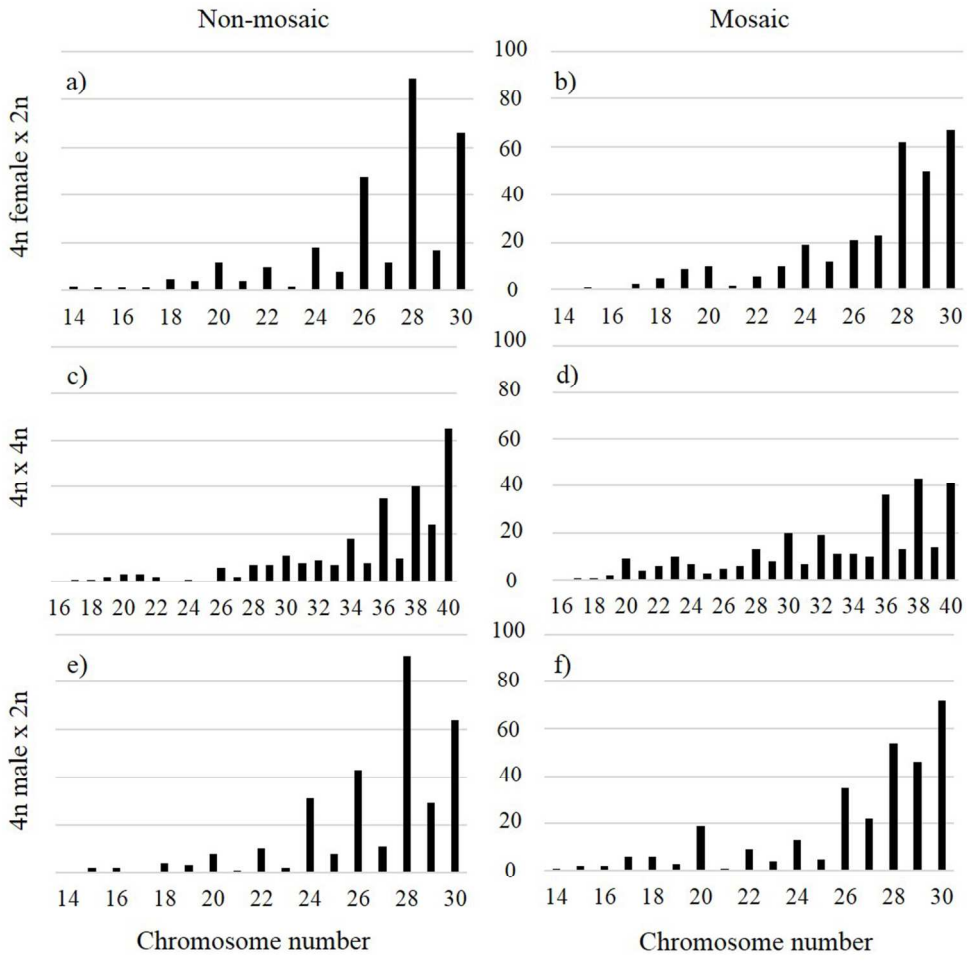
C. virginica juveniles survival estimated at 1 year old the 30 families deployed to the field. Each group of crosses had 10 families. 0 denotes complete mortality in corresponding family. Horizontal bar represents overall average for non-mosaic (solid bars) and mosaic (checkered bars) families. The last 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)



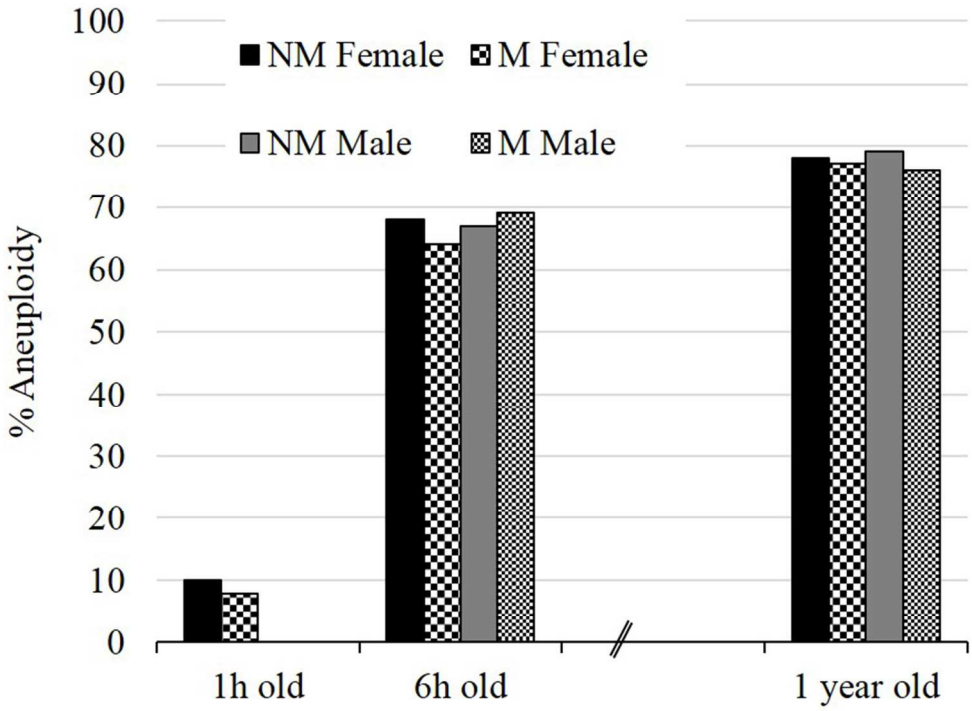
C. virginica juveniles live weight estimated at 1 year old in the 22 out of 30 families that survived through winter. Horizontal bar represents overall average for non-mosaic (solid bars) and mosaic (checkered bars) families.

236x62mm (300 x 300 DPI)



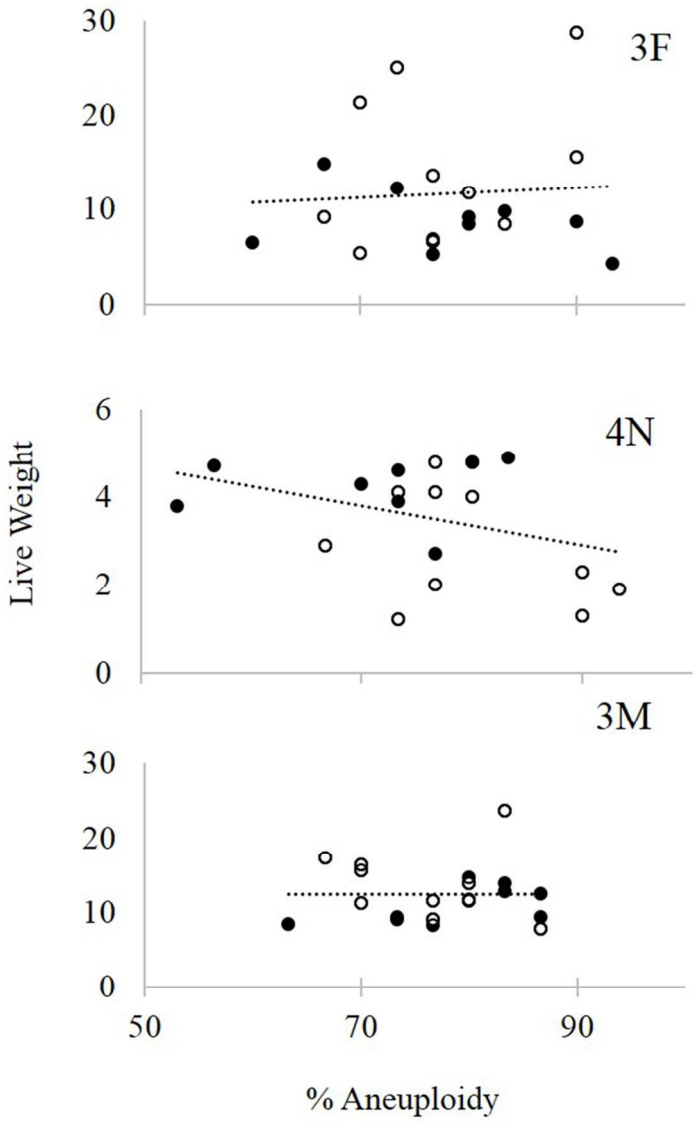
Compiled frequency distribution of chromosome number of cells from triploid C. virginica juveniles produced by crossing either tetraploid non-mosaic females (a) or mosaic females (b) with a diploid male (10 individuals per family), tetraploid non-mosaic males (e) or mosaic males (f) with a diploid female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

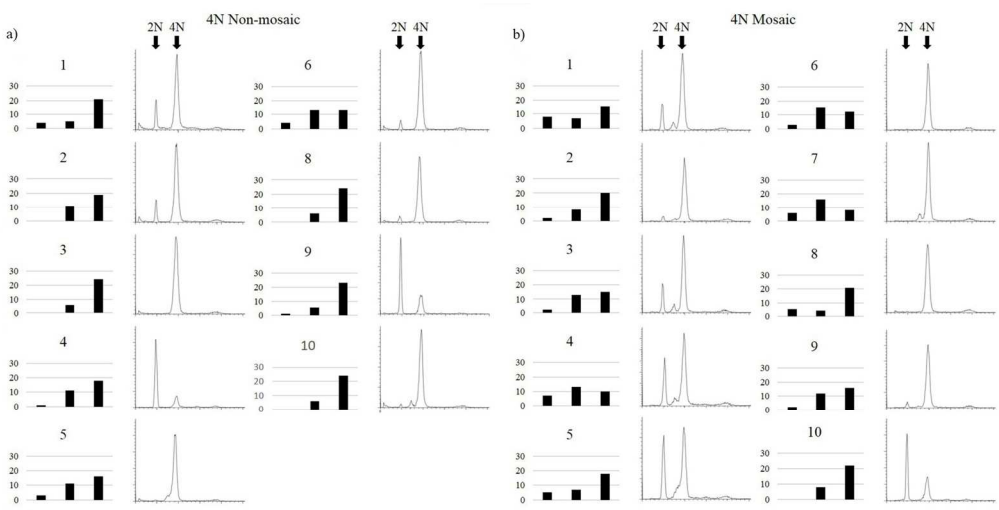


Figure 8. Parallel cytogenetic and FCM data are shown for the tetraploid progeny, obtained by crossing non-mosaic x non-mosaic tetraploids (4N Non-Mosaic, a) or mosaic x mosaic tetraploids (4N Mosaic, b). Chromosome counts were binned into three categories: ≤24 (first bar), 25-34 (second bar), and ≥35 (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)