Heredity (2007) 98, 303–310

www.nature.com/hdy

ORIGINAL ARTICLE

Dosage effects on heritability and maternal effects in diploid and triploid Chinook salmon (*Oncorhynchus tshawytscha*)

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Induced triploidy (3N) in salmon results from a blockage of maternal meiosis II, and hence provides a unique opportunity to study dosage effects on phenotypic variance. Chinook salmon families were bred using a paternal half-sib breeding design (62 females and 31 males) and half of each resulting family was treated to induce triploidy. The paired families were used to test for dosage effects (resulting from triploidy) on (1) the distribution and magnitude of phenotypic variation, (2) narrow-sense heritability and (3) maternal effects in fitness-related traits (i.e., survival, size-at-age, relative growth rate and serum lysozyme activity). Quantitative genetic analyses were performed separately for diploid and triploid family groups. Triploidization resulted in significantly higher levels of phenotypic variance and substantial differences in patterns of variance distribution for growth and survival-related traits, although the patterns were reversed for lysozyme activity. Triploids exhibited higher narrow sense heritability values relative to diploid Chinook salmon. However, maternal effects estimates were generally lower in triploids than in diploids. Thus, the dosage effects resulting from adding an extra set of chromosomes to the Chinook salmon genome are primarily additive. Somewhat counterintuitively, however, the relative magnitude of the combined effects of dominance, epistasis and maternal effects is not affected by dosage. Our results indicate that inheritance of fitness-related quantitative traits is profoundly affected by dosage effects associated with induced triploidy, and that triploidization can result in unpredictable performance and fitness outcomes.
Heredity (2007) **98,** 303–310. doi:10.1038/sj.hdy.6800941; published online 14 February 2007

Keywords: ploidy; growth; survival; phenotypic variance; dosage compensation

Introduction

Induced polyploidy results in a euploid chromosomal state, and hence does not always result in catastrophic genomic imbalance. Polyploidy does increase bulk DNA content, the number of alleles at each locus and, potentially, the interactions among loci. These fundamental changes may modify relationships within (dominance) and between (epistasis) loci, with resultant alterations in gene expression, and ultimately phenotype. Different organisms typically display variable tolerance to polyploidy, with plants generally recognized as more tolerant than animals, and invertebrates more tolerant than vertebrates (Ohno, 1970; Stebbins, 1971). In a number of fish species, induced triploidy is employed to sterilize animals for commercial production, which includes Chinook salmon (*Oncorhynchus tshawytscha*) where the chromosome number increases from 68 (the diploid number) to 102 chromosomes in triploids (Phillips and Rab, 2001).

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Received 17 March 2006; revised 29 August 2006; accepted 23 November 2006; published online 14 February 2007

Induced polyploidy may result in rapid and immediate changes in gene expression mediated by a variety of mechanisms including epigenetic modification, positional effects induced by genomic-level structural changes or transcriptional effects (Adams and Wendel, 2005), mRNA-mediated interference or homology-dependent recognition and gene silencing (Wassenegger, 2002). Modulation of gene expression in triploid genomes may result in gene dosage effects or dosage compensation. When a dosage effect occurs, gene expression is correlated with the number of copies of the gene (ploidy in the case of euploids). Alternatively, dosage compensation may also occur whereby a positive gene dosage effect is compensated for, leading to gene expression at diploid levels regardless of gene dosage levels (Birchler *et al*., 2001).

Evidence from plant and non-vertebrate ploidy series generated using corn (*Zea mays*), fruitfly (*Drosophila melanogaster)* and yeast (*Saccharomyces cerevisiae)* has shown that gene expression in autopolyploids tends to be positively associated with ploidy, that is, a positive gene dosage effect is exhibited (Lucchesi and Rawls, 1973; Birchler *et al*., 1990; Guo *et al*., 1996; Galitski *et al*., 1999). Although some genes were found to have unusually high- or low-expression patterns outside the range of simple gene dosage or dosage compensation effects (e.g., Guo *et al*., 1996) suggestive of epigenetic

mechanisms or transcriptional co-suppression (i.e. negative gene dosage effects), positive gene dosage effects appear to be the most prevalent form of modified expression in ploidy series experiments. However, studies using silkworm (*Bombyx mori*) and cotton (*Gossypium* spp.) ploidy series suggest that a more complex relationship exists between ploidy state, parental origin of chromosome sets and parental-specific regulatory factor influences on expression (Suzuki *et al*., 1999; Adams and Wendel, 2005).

Triploidization can be induced in many salmonid species when a shock (typically heat, pressure or chemical) is applied to a fertilized egg before second polar body extrusion, effectively blocking maternal meiosis II (Benfey, 1999). Despite potential treatment-related trauma (Johnson *et al*., 2004) as well as developmental, cellular, regulatory and phenotypic perturbations related to the retention of an extra set of chromosomes, cellular-level compensatory responses to triploidization are displayed by salmonids and physiological parameters of triploids are remarkably similar to those of diploids (reviewed in Benfey, 1999).

Genetic changes associated with induced triploidy in salmonids have not been thoroughly investigated. Although the increased allelic diversity associated with induced triploidy might be expected to be beneficial (Garnier-Gere *et al*., 2002; Wang *et al*., 2002), triploidy may alter gene interactions or interrupt regulatory factor stoichiometry and epigenetic gene expression patterns resulting in reduced fitness or performance. A comparative quantitative genetic analysis of diploid and triploid offspring would allow a direct estimation of the average phenotypic effect associated with ploidy modification. If complete or partial dosage compensation is occurring in triploids, then heritability (h^2) estimates would be expected to be similar to those of diploids; however, if there is incomplete dosage compensation, then h^2 would be expected to be significantly higher in triploids. Recent published work (Bonnet *et al*., 1999; Blanc *et al*., 2001; Friars *et al*., 2001; Johnson *et al*., 2004) suggests that phenotypic variance is increased in triploid salmon (but see Blanc *et al*., 2005). If all or most gene action is additive, then there should be a linear relationship between phenotypic variance, genetic diversity and additive genetic variation (Reed and Frankham, 2001). Such a relationship would predict higher triploid phenotypic variance and heritability values relative to diploids.

In this study, Chinook salmon were bred using a paternal half-sib mating design to test whether triploidization resulted in changes in (1) the distribution or magnitude of phenotypic variation, (2) narrow-sense heritability (h^2) and (3) maternal effects. Maternal effects occur when the phenotype or genotype of the mother, or the environment she experiences, has a phenotypic effect on her offspring (McAdam *et al*., 2002). Although our analysis is primarily designed to test for changes in the nature of quantitative trait expression in diploid and triploid salmon, the results will have relevance for aquaculture as well. The potential for significant change in the inheritance patterns of performance traits in triploid offspring from a high-performance diploid broodstock has serious implications for the application of triploid sterilization in commercial salmon aquaculture.

Methods

Breeding and rearing

All-female Chinook salmon broodstock from Yellow Island Aquaculture Ltd. (YIAL; Quadra Island, BC, Canada) were mated using a paternal half-sib design. The YIAL broodstock originated from wild Robertson Creek (Vancouver Island, BC, Canada) stock approximately five generations before this project, and has maintained high levels of genetic variation despite little outside broodstock contribution (Bryden *et al*., 2004). In this mating scheme, each of 31 hormonally masculinized phenotypic males (neomales) was mated to two unrelated randomly chosen females (females were bred once and only to one male). The breeding design resulted in a total of 62 full-sib families nested within 31 paternal half-sib groups. The fertilized eggs from each full-sib family were divided into two 250 ml subsamples; one subsample was subjected to a hydrostatic pressure-shock at 6.9×10^4 kPa for 5 min, 30 min after fertilization (to induce triploidy), whereas the other subsample was untreated and transferred to incubation trays immediately after fertilization.

Eggs from each family × treatment group were incubated in separate compartments of vertical stack incubation trays. When eggs reached the eyed stage of development (the point at which the eye spots of the developing embryo are visible (~280–296 accumulated temperature units; ATUs)) they were mechanically shocked and sorted, and live viable eggs were returned to the incubation stacks. Water temperature within the stacks was monitored using a digital data logger (Onset Computer Corp., Bourne, MA, USA) and development stage of the fish was tracked using ATUs. Mean water temperature during the incubation period was 7.72 ± 0.02°C. Mean flow within the stacks was 13 l/min.

As larvae completed yolk-sac absorption (~930–1000 ATUs), 100 larvae were randomly selected from the treatment groups (triploid and control) within each of the 62 full-sib families and transferred to 140 l aerated rearing tanks for the onset of exogenous feeding. Two sets of fish were randomly assigned to each tank at a starting density of 200 fish per tank (~1.43 fish/l). One set of fish in each tank was fin clipped for identification purposes (either the upper or the lower caudal fin lobe was clipped). Flow rate of water to the tanks was approximately 3 l/min and the mean temperature was 8.79°C (7.23–10.18°C). Fish were handfed to satiation multiple times per day with commercial feed (Ewos Canada Ltd., Surrey, BC, Canada).

At approximately 7 months after fertilization, a mean of 55 fish (range 11–116) per family was tagged with coded wire nose tags to identify family and treatment group and transferred to saltwater net cages. The fish were reared under standard commercial rearing conditions until 15 months after fertilization, at which time all fish were humanely euthanized, weighed and the coded wire nose recovered for family and treatment identification.

Ploidy determination

Erythrocyte nuclear length was used to determine family-specific triploidization success (Benfey *et al*., 1984). The validity of this approach has been tested in

Chinook salmon using flow cytometry, where a nuclear length of 8.5 μm was found to be the threshold measurement that most reliably distinguished diploid from triploid individuals (Johnson *et al*., 2004). Fish were euthanized by transfer to a 200 mg l^{-1} tricaine methane sulfonate bath (neutralized and buffered with sodium bicarbonate, pH 7.0). The caudal peduncle was severed and blood was collected in capillary tubes. Whole blood smears were made for approximately 11–20 fish from each family treatment group, and the slides were fixed in methanol and stained with Wright-Giemsa (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada). The length of 10 randomly chosen erythrocyte nuclei per smear was measured to the nearest 0.01 μm, and the mean nuclear length used to determine the ploidy status of each fish. We visualized and measured the erythrocyte nuclei under oil immersion ($\times$1000 magnification) using an Olympus BX-50 compound microscope (Olympus Canada Inc., Markham, ON, Canada) equipped with a QImaging Retiga 1300 Monochromatic digital camera (Quantitative Imaging Corp., Burnaby, BC, Canada) and the Northern Eclipse, version 6.0 imaging program (Empix Imaging Inc., Mississauga, ON, Canada).

Trait measurement

Survival: Incubation survival of family treatment groups was monitored from fertilization to the eyed stage of development, and then followed through to the larval stage, before transfer of the fish to freshwater rearing tanks. Embryo mortalities from fertilization to the eyed egg stage (stage 'S-1') were assessed after the eggs were mechanically shocked and sorted. After the eyed egg stage, incubation survival was determined for all groups at six developmental periods throughout incubation (S-2, S-3, S-4, S-5, S-6 and S-7; see Table 1). Survival of the fry after transfer to rearing tanks (S-8) was determined as the number of live fish remaining immediately before the experimental vaccination treatment divided by the total number of fish originally fin-clipped and released into the rearing tank. Experimentally sacrificed fish within each group (i.e., those fish terminally sampled to determine triploidization success) were excluded from the fry survival assessment.

Table 1 List of measured traits with the number of families (diploid, triploid) and developmental stage in ATUs or days after fertilization

Trait	*Description*	N	*Developmental stage (ATU)*
S-1	Egg survival	62; 62	0–288
S-2	Eyed egg survival	62; 62	288–510
S-3	Larval survival	62; 62	510–616
S-4	Larval survival	60; 62	616–746
S-5	Larval survival	60; 62	746–789
S-6	Larval survival	60; 62	789–853
S-7	Larval survival	60; 62	853–970
S-8	Fry survival	58; 58	970–2008
W-0	Mean fry weight[a]	60; 62	970
W-1	Fry weight	60; 62	1228
W-2	Fry weight	60; 62	1729
W-3	Fry weight	58; 58	1835
W-4	Fry weight	58; 58	1943
W-S	Saltwater weight	56; 46	15 months post-fertilization
RG-t	Fry relative growth, total	58; 58	970–1943
RG-1	Fry relative growth	60; 62	970–1228
RG-2	Fry relative growth	60; 62	1228–1729
RG-3	Fry relative growth	58; 58	1729–1943
SLR	Serum lysozyme response to vaccination	36; 44	2008

Abbreviation: ATU, accumulated thermal units.
[a]Family group pooled weight estimate.

Growth and size-at-age: Wet weight (in g) was determined by sampling fish at five time points during the freshwater (fry) growth of the family groups (W-0, W-1, W-2, W-3 and W-4; see Table 1). Wet weight was also measured at approximately 15 months after fertilization during the saltwater phase of their lifecycle (W-S; see Table 1). At first transfer to the freshwater tanks, 100 larvae from each family were group weighed in water (W-0) as they were transferred to rearing tanks so that a mean family group weight was obtained. Since W-0 did not include individual fish measurements, it was not used as a trait in subsequent analysis, except as a time-zero family weight for relative growth calculations. At all other sample points, weights of individual fish were measured. Approximately 20 fish per family $\times$ treatment group were weighed at the W-1 ($n = 2460$) and W-2 ($n = 2568$) sample points. At the W-3 ($n = 1290$) and W-4 ($n = 1157$) sample points, approximately 10 fish were sampled from each family $\times$ treatment group. At the saltwater sample point (W-S), a mean of 25 diploid fish and 19 triploid fish per family were sampled ($n = 2271$). Blood smears for ploidy determination (see above) were taken from all fish sampled at the W-3 and W-4 sample points.

Relative growth rate of replicate family treatment groups was assessed for the freshwater fry period (after transfer to tanks), a time interval of $\sim$100 days (RG-t in Table 1) but was also calculated for three time periods within the 100 days: RG-1 (32 days), RG-2 (56 days) and RG-3 (29 days; see Table 1). Mean family group weight data were used for the first sampling point (because individual fish were not weighed for W-0), whereas individual fish weights were used for the second sampling point. Relative growth rate for the *i*th fish was calculated as

$$\text{Relative growth}\,(i) = (Y_i - Y_0) \cdot (Y_0 \cdot (t - t_0))^{-1} \cdot 100\%$$

where Y_i is the weight of the *i*th fish at the second sampling point, Y_0 the mean group weight at the first sampling point and $(t-t_0)$ the time interval in days between the first and second sampling points.

Vaccination and serum lysozyme activity: At 6 months after fertilization, approximately 15 fish per family treatment group were euthanized and blood was collected as described above. These fish were used as reference samples for serum lysozyme activity measurements. An additional 15 fish were vaccinated with a commercial vibrio vaccine (Alpha-Dip 2100, *Vibrio anguillarum*, serotype 01 and *V. ordalii* bacterin; Alpharma NW Inc., Bellevue, WA, USA) and sampled as described above, 10 days after vaccination. Vaccine was diluted 1:9 with hatchery water, and fish were immersed for 30 s in

the aerated solution before being returned to rearing tanks. Pre-immunization serum lysozyme activity levels were determined for 769 fish (2N-Control = 379, 3N-Pressure = 390), and post-immunization serum lysozyme activity levels were determined for 842 fish (2N-Control = 390, 3N-Pressure = 452) using the modified microplate assay protocol of Rungruangsak-Torrissen *et al*. (1999) based on Ellis (1993). Briefly, 5 μl of undiluted serum in duplicates for each fish was placed into a well of a 96-well microplate, 95 μl of a 0.21 mg/ml *Micrococcus lysodeikticus* – in 0.05 M phosphate-buffered saline (PBS) solution was then added quickly to all wells using a multichannel pipettor and the absorbance at 450 nm was measured after 1 and 5 min at 25°C using a VERSAmax microplate reader (Molecular Devices, Corp., Sunnyvale, CA, USA). A series of hen egg white controls (4–1000 μg/ml hen eggwhite lysozyme (HEWL) diluted in 0.05 M PBS), blank controls and internal PBS controls were included on each plate. Serum samples with absorbance values outside the range of the HEWL controls were run again in dilution. The enzymatic activity of the HEWL standards was determined using a quality control assay. One unit of lysozyme activity or enzyme unit (EU) was defined as the amount of enzyme causing a decrease in $A_{450\,nm}$ of 0.001/min. The response to vaccination was determined as the individual fish post-vaccination enzyme activity level minus the mean family pre-vaccination enzyme activity level. Sample activity levels were expressed as EU/ml and were $\log_{10}$ transformed to attain normality for statistical analysis.

Statistical analyses

One-way analysis of variance (ANOVA) was used to partition total phenotypic variance for each trait into within- and among-family components. Pitman's procedure for correlated populations was used to test for significant differences between diploid and triploid variances (Zar, 1996). To partition phenotypic variance for the quantitative genetic analysis, nested ANOVA was used with sire and dam factors treated as random effects (Model II). Variance was partitioned among sires, between dams nested within sire, [dam(sire)], and among offspring nested within dam, [offspring(dam)].

Sire additive genetic components (heritability, h^2_{sire}) was estimated using the appropriate mean squares from the nested ANOVAs and variance components calculated using standard formulas as outlined in Roff (1997) and Lynch and Walsh (1998). Standard errors of h^2_{sire} were estimated using the intraclass correlation coefficients following the techniques of Robertson (1959). The sire heritability was used as the best estimate of the additive genetic component because it is not inflated by variance due to dominance or maternal effects and probably only minimally inflated by epistatic effects (Roff, 1997). Sire heritabilities were considered significantly different from zero when the *F*-value derived from the ANOVA indicated a significant sire effect regardless of whether the 95% confidence interval of the h^2 estimate encompassed zero (Roff, 1997).

The paternal half-sib model employed in this study does not allow the decomposition of epistatic, maternal effects or dominance variances. Despite this, a general estimate of the magnitude of maternal effects was calculated using the difference between the dam and sire components of variance divided by the total phenotypic variance for each performance trait. If dominance and/or epistasis are present, it will confound the maternal effects estimate (Roff, 1997). The study design thus allows maternal effects to be detected and generally estimated, but our estimate represents the maximum maternal effect (Roff, 1997).

Analysis was performed separately for diploid control and triploid treatment groups for each trait. Pitman's procedure for correlated populations was used to test significant differences between diploid and triploid maternal variance (maternal effect) estimates (Zar, 1996); however, maternal effects are presented as a percent ratio to total phenotypic variance. Differences between diploid and triploid heritability and maternal effects estimates were examined and a paired sample nonparametric sign test was used to identify the probability of obtaining the observed distribution of differences (Zar, 1996). Since the three early freshwater weight measurements (W1–W3) were highly correlated, we applied the sign test using the mean values of those three values.

Sire and dam heritabilities were estimated for triploidization success (a threshold trait) in pressure-shock treated fish on the underlying or liability (Falconer and Mackay, 1996) scale according to Dempster and Lerner (1950) using Hamaker's (1978) exact approximation of the *z*-value. Standard errors and maternal effects estimates were calculated as above.

Results

Variance among- and within families

Triploidization resulted in increased overall phenotypic variance, although this was driven primarily by among-family variance (Figure 1). Within-family variance for size-at-age did not differ significantly between ploidy types for freshwater size (W-1 to W-4), but differences were observed for saltwater weight (W-S), relative growth (RG) measurements and for serum lysozyme response (SLR; Figure 1a). Triploids exhibited significantly higher among-family phenotypic variance than diploids for most traits with the exception of one incubation survival estimate (S-4), one measure of relative growth rate (RG-3) and SLR (Figure 1b).

Quantitative genetics

A number of interesting differences between diploids and triploids were noted in the partitioning of phenotypic variance among sires, dams nested within sires, and progeny within dams in the sib analysis and the estimation of heritability (Table 2; see Supplementary Table 1). Results are reported in terms of the distribution of variance, the relative contribution of causal components along with the heritability and maternal effects estimates (Table 2; see Supplementary Table 1).

Size-at-age (W-1 to W-4; WS): The sire component of variance in size-at-age was considerably smaller than the dam component (nested within sires) for all but one trait (W-S in triploids) in both diploids and triploids (see Supplementary Table 1). However, the sire component of variance in triploids was, in all cases, substantially larger

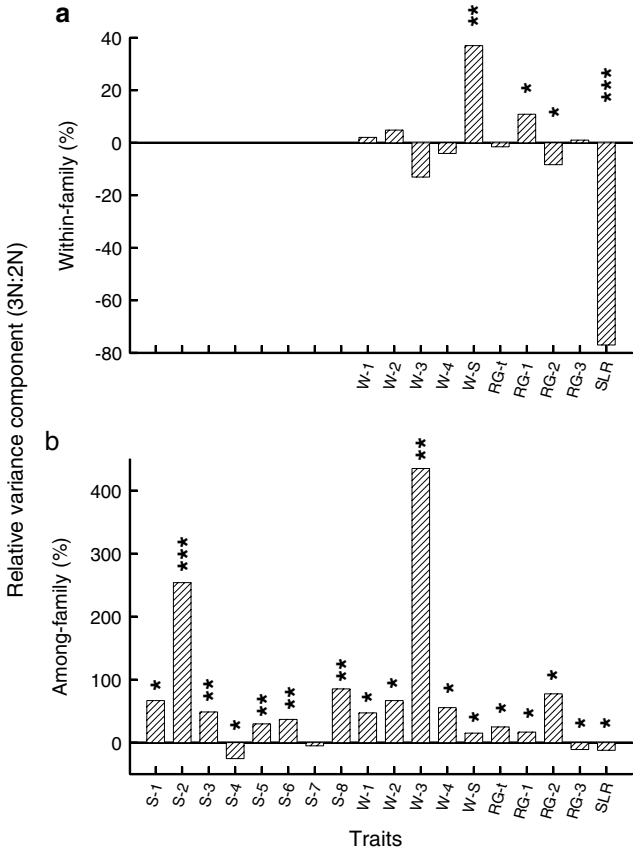

Figure 1 Relative variance components (triploid versus diploid) for size (W-1 to W-4 and W-S), growth (RG-t and RG-1 to RG-3), survival (S1–S8) and SLR to vaccination traits in Chinook salmon. Values were calculated as percent difference between triploid and diploid values relative to the diploid absolute value. (**a**) Within-family variance components (not estimated for survival traits), (**b**) among-family variance components. $*P \leqslant 0.05$, $**P \leqslant 0.01$ and $***P \leqslant 0.001$.

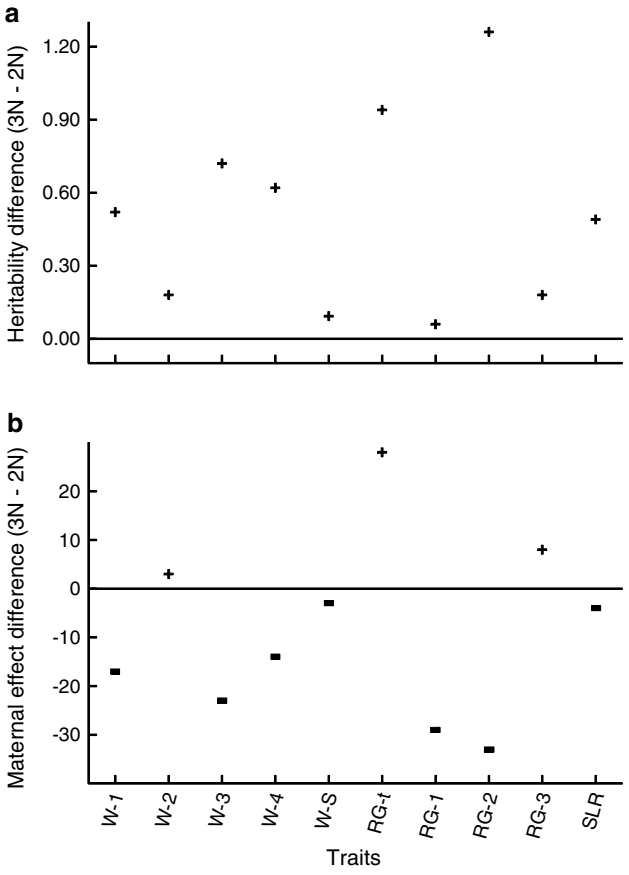

Figure 2 Difference between diploid and triploid heritability and maternal effects estimates for size (W-1 to W-4 and W-S), growth (RG-t and RG-1 to RG-3) and SLR to vaccination (SLR) traits in Chinook salmon. (**a**) Heritability differences for triploid versus diploid families are marked with a plus sign if $h^2_{\text{triploid}} > h^2_{\text{diploid}}$, no traits showed $h^2_{\text{triploid}} < h^2_{\text{diploid}}$. (**b**) Maternal effect estimate differences for triploid versus diploid families are marked by a plus sign if $V_{\text{m(triploid)}} > V_{\text{m(diploid)}}$ and with a minus sign if $V_{\text{m(triploid)}} < V_{\text{m(diploid)}}$.

Table 2 Sire component heritability ($h^2 \pm$s.e.) and maternal effect estimates (V_m, expressed as % relative to total variance) for measured traits in diploid and triploid families

Trait	$h^2_{sire} \pm s.e.$		V_m *% relative*	
	Diploid	*Triploid*	*Diploid*	*Triploid*
W-1	0.50±0.21	1.02±0.35*	44**	27
W-2	−0.32±0.10	−0.14±0.29	62	66***
W-3	−0.01±0.07	0.71±0.29	51***	28
W-4	0.18±0.14*	0.80±0.33*	38***	24
W-S	−0.003±0.02	0.09±0.02**	1.2	−2.8
RG-t	0.10±0.11	1.04±0.31**	46	74***
RG-1	0.13±0.08	0.19±0.11	29	0
RG-2	−0.07± 0.02	1.19±0.39***	33	0
RG-3	0.13±0.12	0.31±0.18	0	8
SLR	−0.41±0.10	0.08±0.15	43***	31
Triploid rate	NA	0.32±0.14	N/A	13

Heritabilities significantly different from zero are marked with asterisks, as are significant differences between diploid and triploid raw maternal variance estimates (relative values presented). $*P \leqslant 0.05$, $**P \leqslant 0.01$ and $***P \leqslant 0.001$.

than the comparable diploid value (see Supplementary Table 1). Thus, the estimates of heritability (h^2) were consistently higher in triploids at each sample point (Table 2; Figure 2a). However, the large standard errors associated with the heritability estimates for size-at-age result in 95% confidence intervals that rendered differences between diploid and triploid estimates predominantly non-significant. Maternal effects estimates for size-at-age were mostly substantially lower for triploids than for diploids at each sample point (Table 2; Figure 2b).

Relative growth rate (RG-t, RG-1, RG-2, RG-3): Triploids exhibited substantially more among-sire variance for RG-t than was observed in diploids. Among-sire variance accounted for 7% of the total phenotypic variance in triploids and only 2% in diploids; this resulted in a much larger RG-t heritability in triploids than in diploids (Table 2; Figure 2a). The distribution of variance among dams and among progeny was substantially different between ploidy types in terms of magnitude (2N: 49% among dams, 49% among progeny; 3N: 81% among dams, 12% among progeny; see Supplementary Table 1), suggesting that there was more intra-family variation in diploids,

but potentially more influence of dominance, epistasis and maternal effects on RG-t in triploids than in diploids. The relative maternal effects estimates (Table 2; Figure 2b) supported the higher influence of the maternal effects component in triploids for RG-t.

The general trends in variance distribution indicate that triploid relative growth rate was becoming more variable over time whereas diploid variability declined. Diploids and triploids exhibited very different temporal patterns in the distribution and magnitude of variance components for the stage-specific relative growth rates (RG-1, RG-2 and RG-3; see Supplementary Table 1). Heritability was quite variable for relative growth rate in triploid fish, but was fairly constant for diploid groups (Table 2; Figure 2a). Relative maternal effect estimates (V_m) were substantially lower in triploids than in diploids in the first two growth periods (RG-1 and RG-2; Table 2; Figure 2b).

Serum lysozyme response: The phenotypic variance components of SLR to vaccination were similar in diploids and triploids (see Supplementary Table 1). There was no among-sire variance in diploids and very little in triploids, and the remaining phenotypic variance was predominantly distributed at the among-progeny level, although among-dam variance represented 30 and 33% of the total variance in diploids and triploids, respectively. Heritability estimates of the response to vaccination in diploids and triploids did not differ significantly from zero or from each other (Table 2; Figure 2a); however, relative maternal effect estimates for the response to vaccination were higher for diploid families (Table 2; Figure 2b).

Triploidization success: Variance in triploidization success was partitioned mainly among progeny (65%) and dams (24%; see Supplementary Table 1). The underlying heritability estimate, while not associated with a significant sire effect, was moderate and the 95% confidence interval did not encompass zero (Table 2). Maternal effects were present but were relatively low, suggesting that the combined environmental and genetic components of the effect of the maternal phenotype, dominance and epistatic effects on triploidization of offspring were not large.

Sign test of heritability and maternal effect differences: Triploid heritabilities were consistently higher than those of diploids (Figure 2a) and the sign test confirmed this by rejecting the null hypothesis that the median difference between triploid and diploid heritability estimates was zero ($P<0.03$). Although triploid maternal effects were generally lower than diploid estimates (Figure 2b), the sign test did not reject the null hypothesis that the median difference between triploid and diploid estimates was zero ($P>0.05$).

Discussion

Given the genetic and environmental stress associated with pressure-induced triploidization, it is perhaps not surprising that total phenotypic variance is elevated in triploid relative to diploid Chinook salmon. The dramatically elevated among-family phenotypic variance associated with triploidy, however, indicates that the addition of an extra set of chromosomes must enhance family trait differences. We expected dosage effects, if present, to have a greater effect on the among-family variance component, relative to the within-family variance component. This is because the additional set of maternal chromosomes would have a limited effect on within-family variation (where all offspring are approximately equally affected), but would exaggerate among-family mean trait differences, thus leading to inflated among-family variance. Increased among-family phenotypic variation in triploids for growth- and survival-related traits, therefore, signifies dosage effects in those traits. Curiously, dosage effects were not seen in the lysozyme response to vaccination. We do not know why dosage effects are apparent for phenotypic variance in the growth- and survival-related parameters but not in the immune parameter, although it may reflect differences in the regulatory control complexity of growth-related genes compared with non-specific immune genes. Significantly elevated among- and within-family phenotypic variance in growth parameters (length and weight) was recently reported for triploid full-sib Atlantic salmon families (Friars *et al*., 2001). Those authors attributed the higher levels of variance to a triploidization-induced disruption of uniformity, but did not hypothesize a mechanism. Blanc *et al*. (2005) reported higher phenotypic variance for growth-related traits in diploid offspring in their among-sire analysis, but higher triploid offspring variance in their among-dam analysis in brown trout (*Salmo trutta*). However, since Blanc *et al*. (2005) used pooled gametes for their mating experiments, their variance estimates are pooled among- and within-family variance. Suzuki *et al*. (1999) investigated gene transcription levels in diploid and triploid silkworms, and found that dosage effects versus compensation varied with the gene studied. Perhaps the dosage effects resulting from polyploidy may vary considerably among individual genes, but highly polygenic traits reflect a genome-averaged effect. The equivocal reports of dosage effects on phenotypic variance in ploidy-manipulated organisms suggest that the effect of polyploidy may vary with species and/or mating design.

In this study, heritability estimates were significantly higher in triploids than in diploids (see Figure 2a). An increase in gene dosage is expected to cause increases in the observed phenotypic variance due to elevated additive genetic variance if there is a linear relationship between the level of gene transcription and its effect on phenotype. Hence, if dosage compensation does not occur, high triploid heritability for growth and survival traits that typically show moderate heritability in diploids (Gjedrem, 1983) would be expected. We observed such a pattern, and hence conclude that genome-wide ploidy-dependent regulation (i.e., positive gene dosage effects) of gene expression averaged over all quantitative growth-related gene loci appear likely in Chinook salmon. Although elevated heritability associated with an increase in ploidy is likely a result of increased additive genetic variance due to dosage effects, it is also possible that heritability may be inflated owing to an increase in epistatic interactions (Cheverud and Routman, 1995). These interaction terms are generally ignored in calculations such as ours because they are expected to be very small (Falconer and Mackay, 1996;

Roff, 1997); however, triploids have unknown additive-by-additive interactions. It is possible that dosage effects could drastically affect such epistatic effects, especially since the additive effect of a gene will change depending on the frequencies of its epistatic partners (Cheverud and Routman, 1995; Wade, 2002).

Maternal effects are defined as the non-genetic influences of the maternal phenotype, genotype and environment on the phenotype of the offspring (Falconer and Mackay, 1996). Maternal effects in Chinook salmon are mainly transmitted through egg allocation, as there is no maternal care (Heath and Blouw, 1998). As among-dam variance in paternal half-sib experimental designs (such as this) includes additive genetic variance components, maternal effects and higher-order interaction effects, it is generally not considered useful for estimating narrow sense heritability. However, it can be used to estimate maternal effects by subtracting the among-sire variance from the among-dam (nested within sire) variance and expressing it as a proportion of the total variance (Falconer and Mackay, 1996). Such an approach may not be entirely appropriate for triploid offspring since the relative contribution from the confounding dominance and epistatic effects is simply unknown; however, maternal effects are known to be very large for diploid Chinook salmon fry and are expected to swamp the non-additive genetic variance components during early development (Heath *et al*., 1999).

Interestingly, maternal effects estimates were found to be lower for triploids than for diploids for most traits (Figure 2b). This is especially interesting because extra copies of maternal genes would be expected to inflate the influence of epigenetic maternal effects and thus magnify our maternal effects estimates. This clearly is not the case. Generally, the absolute maternal effects values in triploids exhibited a trend toward lower values ((dam variance–sire variance); see Supplementary Table 1), indicating that the pattern is neither trait-specific, nor a calculation artifact. Early life growth and survival traits are known to be strongly influenced by maternal effects in salmon (Heath and Blouw, 1998; Nagler *et al*., 2000); however, the magnitude of growth-related maternal effects in Chinook salmon decreases as juveniles develop, becoming not significantly different from zero by $\sim$150 days after fertilization (Heath *et al*., 1999). In our study, we observed a loss of maternal effects in relative growth over time in the diploid offspring, but not in the triploid offspring (which remained essentially zero). Thus, the triploid maternal effect estimates for relative growth likely do not include an appreciable maternal effect component, nor do they apparently reflect dramatic phenotypic effects of dominance and epistasis. We did not observe any obvious temporal pattern for maternal effects on size at age (W-1 to W-4), although an increase in relative V_m appeared at the W-2 stage. Such an increase is not consistent with the expectation of monotonic decreases in maternal effects through development (Heath *et al*., 1999), and may reflect dominance and epistatic effects. Maternal effects were negligible in both diploid and triploid families by 15 months after fertilization (W-S). If the main effects of induced triploidy on phenotype are additive due to the increased dosage of nuclear genes, the relative effects of dominance and epistatic interactions might not be detected.

We observed a few apparently anomalous estimates of variance, heritability and maternal effects among our time-series measures of similar traits. The elevated relative within-family variance component for saltwater weight (W-S, Figure 1a) likely reflects the highly variable saltwater environment and the reported variable performance of triploid fish under such conditions. The elevated S-2 and W-3 among-family variance estimates are more difficult to explain; although the developmental period encompassing S-2 (the eyed egg stage) often has highly variable triploid family survival, W-3 is within a stable growth period for both triploid and diploid salmon, given that it closely precedes smoltification (saltwater adaptation). The variation in magnitude of the heritability differences for the various traits probably reflects the relatively large error associated with h^2 measures, even when based on 50 or more half-sib families.

An important result of our study was that triploidization success was shown to have a moderate additive genetic basis ($h^2 \pm \text{s.e.} = 0.32 \pm 0.14$). This is the first estimate of heritability reported for such a trait and indicates that there is likely a genetic component to the cellular response to pressure-shock in Chinook salmon. Thus, a breeding program to improve triploidy rate should be successful. The distribution of the variance components in the sib-analysis of triploidization success was unexpected as the majority of variance was attributable to differences among progeny rather than differences among dams or sires. This was surprising since all of the eggs from a single female were subjected to the pressure-based triploidization treatment simultaneously. Since the application of hydrostatic pressure within the cylindrical pressure chamber would be uniform, and there is minimal egg-size variation within a maternal family, high inter-individual variation in triploidization success within dams likely indicates significant differences in the susceptibility of individual eggs to polar body retention. Such differences can be most easily explained by differences in the timing of meiosis II among individual gametes within a clutch. While family differences in triploidization success in salmonids has often been noted (Teskeredzic *et al*., 1993; Galbreath and Samples, 2000), egg-level variation in meiotic timing, microtubule structure or egg provisioning have not been previously suggested.

In summary, triploidization increased phenotypic variation for growth and survival traits in Chinook salmon. Additionally, the proportion of phenotypic variance attributable to the average additive effects of alleles (additive genetic variance and heritability estimates) increased after triploidization, whereas the relative size of dominance, epistatic and maternal effects did not. This pattern of variance distribution indicates that the primary effects of adding an extra set of chromosomes to the salmonid genome are additive and heritable. Dosage effects are thus present in triploid Chinook salmon and we predict that specific gene expression patterns will show a general upregulation in triploids. Since triploidization appears to disrupt the normal inheritance of performance gains made through the selective breeding of diploids, the utility of triploids for aquaculture purposes may be compromised. The potential ecological and financial benefits of producing sterile triploid stock, however, must be weighed against their potential genetic disadvantages.

Acknowledgements

Financial support for this work was provided by Yellow Island Aquaculture Ltd., the Science Council of BC (Technology BC) and the Natural Sciences and Engineering Council (NSERC) of Canada Collaborative Research and Development program. RMJ was supported by an NSERC Industrial Postgraduate Scholarship. We also thank JW Heath, VA Heath, A Clarke, G Osbourne, C Richardson, A Newhook, M Stuyt, H Robert, R Beecroft (ImmunoPrecise Antibodies) and D Stanton for assistance with field sampling and laboratory analyses.

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