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Effects of polyploidy and reproductive mode on life history trait expression

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EFFECTS OF POLYPLOIDY AND REPRODUCTIVE MODE ON LIFE HISTORY TRAIT EXPRESSION

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CERTIFICATE OF APPROVAL

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MASTER'S THESIS

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This is to certify that the Master's thesis of

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ABSTRACT

Although genomes are an important element of living systems, why they feature such striking variation and how this variation is maintained within and across natural populations remains unclear. One of the most common and important means by which genomic variation is generated is ploidy elevation. While polyploidy has been implicated in the remarkably successful radiations of angiosperms, teleost fish, and amphibians, the phenotypic consequences of changes in ploidy level are poorly understood, especially in animals. I use a large, multi-year common garden experiment to identify potential life history costs and benefits of polyploidy and asexual reproduction, a trait often associated with polyploidy, in *Potamopyrgus antipodarum*. This snail is well suited for studying ploidy variation and sex because diploid sexuals and triploid and tetraploid asexuals frequently coexist, allowing us to use comparisons of sexuals to asexuals and triploid to tetraploid asexuals to study both the effects of ploidy elevation and sex. I detected a strong negative correlation between growth rate and time to maturity and found that sexual *P. antipodarum* grew and matured significantly more slowly than the polyploid asexuals. Sexual *P. antipodarum* were also more likely to die before achieving reproductive maturity than their asexual counterparts. By contrast, there were no apparent life history differences between triploid and tetraploid asexuals, indicating that direct phenotypic benefits of ploidy elevation are unlikely to explain the relatively rapid growth and maturation of asexuals. My results suggest that ploidy elevation does not inevitably confer phenotypic consequences, that reproductive mode influences life history trait expression, and that sexual *P. antipodarum* persist in many natural populations in spite of substantial life history disadvantages.

PUBLIC ABSTRACT

Why nuclear DNA content varies so much within and among species remains one of the major unanswered questions in biology. One of the most common means by which DNA content can change is ploidy elevation, the addition of one complete set of chromosomes relative to the normal number of chromosome sets in a species. Even though ploidy elevation is common, we still know very little about how extra chromosome sets affect organismal biology. Here, I studied the effects of ploidy elevation in a snail system, *Potamopyrgus antipodarum*. These snails are ideally suited for studying the effects of ploidy elevation because snails with 2, 3, or 4 sets of chromosomes ("2x", "3x", "4x") coexist in nature, allowing me to use direct comparisons between otherwise similar snails that differ in ploidy to identify effects of ploidy elevation. I focused on three traits, growth rate, age at reproduction and adult body size, that are all main determinants of reproductive fitness in *P. antipodarum*. I found that 2x snails grow more slowly and reach reproductive maturity later than 3x and 4x snails and that 2x snails were more likely to die before reproducing. I did not detect any differences in any of these traits between 3x and 4x snails. One explanation for the lack of differences between 3x and 4x snails is that the differences between the 2x and 3x/4x snails were driven by reproductive mode (2x snails reproduce sexually while 3x and 4x snails reproduce asexually) rather than ploidy elevation.

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INTRODUCTION

There is a growing body of evidence that polyploidy has played a key role in the diversification of angiosperms (e.g., *Amborella* Genome Project 2013, Vanneste *et al*. 2014) and other eukaryotic taxa (Van de Peer *et al*. 2009). Increased recognition of the importance of polyploidy has inspired research highlighting the likelihood that ploidy elevation will often confer major phenotypic (reviewed in Ramsey and Ramsey 2014; e.g., Neiman *et al*. 2009, Arvanitis *et al*. 2010, Balao *et al*. 2011) and genomic (e.g., Hollister *et al*. 2012, Martin and Husband 2012, Yant *et al*. 2013) consequences. This research has demonstrated that polyploidy can influence many important individual and population-level traits, ranging from likely positive effects such as increased enzymatic activity (Levin 1983), higher levels of gene expression (De Godoy *et al*. 2008, Neiman *et al*. 2009), and greater adaptive potential (Otto and Whitton 2000, Balao *et al*. 2011, Martin and Husband 2012) to costs associated with ploidy effects on organismal ecology (e.g., increased sensitivity to nutrient limitation, Neiman *et al.* 2013a) and genomic mutational load (Otto and Whitton 2000). Despite all of these discoveries, the overall biological significance of polyploidy remains unclear (Otto and Whitton 2000, Mable *et al*. 2011, Choleva and Janko 2013, Leslie 2014).

Until recently, polyploidy was thought to be so rare amongst animals that it was generally inconsequential (Mable 2004, Mable *et al.* 2011). The frequent presumption that animal polyploids are uncommon, inviable, and/or infertile is likely to explain both why most studies of the consequences of polyploidy have focused on plants and why the phenotypic consequences of polyploidy in animals remain largely unknown (Mable 2004). Here, I use a powerful natural animal system to address whether and to what extent polyploidy influences life history traits like growth rate, body size, and age at reproductive maturity. Life history traits are an appropriate and

important focus for the study of the ecological and evolutionary importance of polyploidy because 1) life history traits are important determinants of fitness (e.g., Levy and Feldman 2002, Otto 2007, Ramsey 2011), and 2), ploidy level has the potential to influence life history traits in both positive and negative ways (e.g., Levin 1983, Cavalier-Smith 1978). For example, if polyploids have higher per-organism gene expression (e.g., De Godoy *et al*. 2008, Neiman *et al*. 2009), the positive correlation between RNA level and growth rate (Hessen *et al*. 2010) means that polyploids might be expected to grow more rapidly than diploids (Neiman *et al*. 2013b). Conversely, polyploidy might be associated with relatively slow growth and maturation if increased per-cell DNA content causes decreased metabolic rate (Cavalier-Smith 1978, Hessen *et al.* 2013) and/or increased cell cycle duration (Davies and Reese 1975, Bennett and Leitch 2005, Gregory 2005).

Potamopyrgus antipodarum, a New Zealand freshwater snail, is well suited to empirically evaluate connections between ploidy level, reproductive mode, and life history variation because it combines extensive variation in key life history traits (Jakobsen and Forbes 1997, Jokela *et al*. 1997a, Jokela *et al*. 1999, Jensen *et al*. 2001, Neiman *et al.* 2013b, Krist *et al.* 2014) with widespread within- and across-population ploidy polymorphism (diploids, triploids, and individuals that exceed triploidy; hereafter "tetraploids"; "2x", "3x", "4x", respectively) (Neiman *et al*. 2011, Paczesniak *et al.* 2013). Like many other polyploid animals (reviewed in Otto and Whitton 2000, Neiman and Schwander 2011), polyploid *P. antipodarum* are asexual (Phillips and Lambert 1989). The existence of triploid and tetraploid asexual *P. antipodarum* means that I can use comparisons between asexuals of different ploidy levels as well as between sexuals and asexuals to simultaneously study the consequences of polyploidy and identify costs and benefits associated with sexual *vs*. asexual reproduction, another key unanswered question in

biology. An additional strength of the *P. antipodarum* system in this context is that, unlike many polyploid animals and plants (Bierzychudek 1985, Otto and Whitton 2000, Neiman and Schwander 2011), triploid and tetraploid *P. antipodarum* are not hybrids. Rather, polyploid *P. antipodarum* are the products of multiple separate ploidy elevation events from lower ploidy *P. antipodarum* (Neiman *et al*. 2011, Paczesniak *et al.* 2013), likely via occasional fertilization of sexual diploids (producing triploids) and asexual triploids (producing tetraploids) by sympatric males (Neiman *et al.* 2011). The implications are that each different triploid and tetraploid *P. antipodarum* lineage constitutes a distinct natural experiment into the consequences of ploidy elevation and the absence of sex.

Here, I use a common garden approach to evaluate whether the means and variances of growth rate, time to reproductive maturity, and adult female body size differ across ploidy levels and reproductive modes. I chose to focus on these traits because they are all likely to be important determinants of fitness in female *P. antipodarum* (Krist and Lively 1998, Tibbets *et al*. 2010, McKenzie *et al*. 2013). A positive association between ploidy level and one or more of these traits would be an exciting discovery, indicating that ploidy elevation confers life history benefits in a natural animal system. This results would also suggest that asexual *P. antipodarum* are likely to have, at least in some environments, an even greater than the generally assumed two-fold advantage that asexuals experience relative to otherwise similar sexuals (Maynard Smith 1978, Jokela *et al.* 1997b). Such a result would also emphasize the likelihood that the selective maintenance of diploid sexual *P. antipodarum* is a consequence of direct benefits of sex (e.g., recombination). By contrast, decreased growth and/or maturation rate as ploidy level increases would implicate costs of polyploidy as a potential explanation for the relative scarcity of tetraploid *vs*. triploid asexual *P. antipodarum* (Neiman *et al.* 2011) and as a potential

contributor to the success of sexual *P. antipodarum.* I also used my data to evaluate one of the most fundamental components of many hypotheses for sex as well as an expected advantage of ploidy elevation: that sex, via recombination, and ploidy elevation, via the availability of extra allelic copies (Otto and Whitton 2000), will increase the phenotypic variance of heritable traits among sexually produced offspring relative to asexually produced offspring (Barton 1995) and in higher ploidy *vs*. lower ploidy offspring.

METHODS

Animals

I initiated the experiment with a total of 169 adult (>3 mm) female *P. antipodarum* ("founding females") isolated from either field collections or laboratory cultures between January 2011 and May 2011. 140 of the founders, representing 20-40 females collected from each of six New Zealand lakes, were sampled from their native lake in January 2011 (Table 1). I selected lakes that allowed us to maximize the probability that I would sample multiple *P. antipodarum* of more than one ploidy level from the same lake, allowing us to perform comparisons without the introduction of the potentially confounding effects of lake of origin. With the exception of Alexandrina *Isoetes* snails, all *P. antipodarum* were sampled from shallow locations (<2 meters in depth). The Alexandrina *Isoetes* individuals were sampled from depths of ~2-4 meters. In order to increase the genetic and geographic diversity of the *P. antipodarum* in the experiment, I also included one founding female from each of 14 triploid and 15 tetraploid asexual laboratory cultures ("lineages") (characterized in Neiman *et al.* 2012). These lineages were descended from single females originally collected from nine different New Zealand lakes in January 2009 (Neiman *et al*. 2011, Neiman *et al*. 2012; Table 1).

Snail Housing and Care

All founding females were individually housed in a one-liter cup filled with ~200 mL carbon-filtered tap water and fed three times per week with dried *Spirulina*, a common laboratory food source for *P. antipodarum* (e.g., Neiman *et al*. 2013c, Zachar and Neiman 2013). All females isolated from the five lakes known to have a relatively high frequency of diploid sexuals (>10% sexuals; Neiman *et al*. 2011, Paczesniak *et al*. 2013; Table 1) were housed with males to ensure

that sexual females, which are phenotypically indistinguishable from asexual females, would have the opportunity to become fertilized. Because many of these males were field collected and thus subject to infection by sterilizing trematode parasites (Winterbourn 1973, Lively 1987), I rotated males through all cups from these five lakes on a bi-weekly basis to ensure that all females had access to fertile males. Because these parasites are sterilizing, any female that was infected was excluded from all subsequent analyses.

Isolation of offspring

I checked the cup of each founding female under a dissecting microscope three times per week for newly born offspring (G1), recorded the date each G1 was found, and then removed and housed each G1 individually in its own cup. This process was repeated for the first eight G1 offspring produced by each founding female. I snap-froze and stored at -80°C one additional offspring produced by each founding female, to be used later for flow cytometric determination of nuclear DNA content (*i.e*., ploidy; e.g., Neiman *et al.* 2011).

Measuring growth and reproductive maturity

I checked the G1 cups once per week until the shell of the G1 calcified. At this point, the shell was visible to the naked eye and the G1 was ~ 1.0 mm in length. I then used a camera and a dissecting microscope to capture an image of the snail next to a ruler while it was crawling along the bottom of a petri dish. Next, I imported this image into ImageJ and measured shell length from apex to aperture for each snail. I repeated this process weekly until the G1 reached 3.0 mm in shell length, ~0.5-1.5 mm below the shell length at which reproduction typically commences in female *P. antipodarum* (Winterbourn 1970, Tibbets *et al.* 2010, McKenzie *et al.* 2013; present

study). At this point, I began rotating males through the cup for G1 snails from the five lakes with sexuals and checking all cups three times a week for offspring (G2). Upon finding a G2 (mean 109.103 +/- SD 64.055 days after the 3.0 mm threshold), I recorded the date of birth (accurate within 2-3 days; hereafter, "age at maturity"), and then used a dissecting microscope, camera, and ImageJ software to measure the shell length of the now-reproductively active G1. *Potamopyrgus antipodarum* growth slows markedly at reproduction, and *P. antipodarum* female fecundity is strongly and positively associated with shell length (Winterbourn 1970, Tibbets *et al*. 2010, McKenzie *et al.* 2013), meaning that shell length at first reproduction (hereafter, "final length") serves as a meaningful reproductive fitness correlate. Throughout this process I performed weekly mortality checks on all G1 snails and recorded the date of death (accurate within a week) for all dead individuals.

Flow cytometry

Tissue samples for flow cytometry were prepared following Neiman *et al*. (2011, 2012). I used head tissue in individuals >3.0 mm in length and tissue from entire individuals that were <3.0 mm in length. I ran the samples on a Becton Dickinson LSR II flow cytometer. A 20 ul sample of chicken red blood cell standard (Lampire Biological Labs, Pipersville, PA) was prepared in the same manner as the snail tissue and was run at the beginning of each flow cytometry session in order to calibrate the machine so the DAPI-A peak was centered at 80 FL1 units. I then used the FL1 channel to measure the DAPI fluorescence of each nucleus, which indicates DNA content and thus ploidy level, and used FlowJo software (Version 8.8.7, Tree Star, Inc.) to analyze the flow cytometry data.

Statistical Analyses

I began by addressing the primary question of whether ploidy level affected my three focal life history traits: growth rate (represented as growth (mm) per day until 3.0 mm in shell length) for G1 females, age at maturity for G1 females, and final length for G1 females. I first used the Kolmogorov-Smirnov (K-S) test to evaluate whether each of the three dependent variables met the normal distribution requirement of parametric statistical analysis. While final length data were distributed normally (K-S statistic = 0.041, df = 302, $p = 0.200$), the growth rate (K-S statistic = 0.073, df = 302, $p = 0.001$) and age at maturity data (K-S statistic = 0.099, df = 302, $p < 0.0001$) exhibited significant deviations from normality. After a natural log transformation, the age at maturity data (K-S statistic = 0.040, df = 351, $p = 0.200$) were distributed normally. I was able to achieve a normal distribution with the growth rate data following a cube root transformation (K-S = 0.034, df = 365, $p = 0.200$), allowing me to use parametric analyses for all three life history variables for all subsequent analyses.

The existence of a size threshold at which *P. antipodarum* females first start reproducing (Winterbourn 1970, McKenzie *et al.* 2013; present study) suggests that variation in growth rate might be associated with variation in age and size at reproductive maturity. With this logic in mind, I used linear regression analyses to evaluate whether I would need to correct for effects of growth rate on age at maturity and final size, respectively.

Growth rate was significantly associated with both age at maturity (linear regression; beta = -0.652, $F = 237.130$, $p < 0.0001$) and final length (linear regression; beta = 0.221, $F = 15.488$, $p < 0.0001$), so I saved the residuals from these regression analyses as growth rate-corrected estimates of age at maturity and final length. I then used these residuals (along with growth rate itself) as dependent variables in general linear model analyses evaluating whether the fixed

factor of ploidy level influenced the dependent variables of growth rate, age at maturity, and final length. I controlled for descent from the same founding female by nesting the random factor of family, defined as all G1 *P. antipodarum* that descend directly from the same founding female, within ploidy level, and used post-hoc Tukey's honestly significant difference analyses to determine whether there were differences in growth rate, age at maturity, and/or final length between particular ploidy levels. Because shell length in female *P. antipodarum* is positively associated with fecundity (Winterbourn 1970, McKenzie *et al*. 2013), I also conducted these same analyses with the raw (*i.e*., non-growth rate-corrected) final length data. While the outcome of these analyses could be driven by the positive association between growth rate and final length (see above), they also provide me with a straightforward means of assessing whether shell length and thus fecundity differs across ploidy levels and/or reproductive modes. By similar logic, because age at maturity (regardless of associations with growth rate) is likely to be an important determinant of reproductive success prior to, for example, parasitic sterilization (Jokela and Lively 1995) and/or predation (Levri 1998), I also performed a set of analyses using the raw age at maturity data.

Because my regression analyses suggested that variation in growth rate might be an important determinant of age at maturity and final length, I used correlation analyses to evaluate whether and how growth rate was correlated with the other two variables. In order to account for non-independence of family members, I first calculated mean family values for each of the three life history variables from the individual-level data. I then used the K-S test to evaluate whether these family means were normally distributed. None of the datasets exhibited significant deviations from normality (growth rate: K-S statistic = 0.056, df = 95, $p = 0.200$; age at maturity: K-S statistic = 0.059, df = 95, $p = 0.200$; final length: K-S statistic = 0.065, df = 95, $p = 0.200$),

so I used the parametric Pearson's r approach for both correlation analyses.

The maintenance of intraspecific life history variation is often linked to spatially variable selection (recently reviewed in Richardson *et al*. 2014), and the existence of substantial genetic variation for all three life history traits within *P. antipodarum* (present study; also see Jokela *et al.* 1997a, Jacobsen and Forbes 1997, Jensen *et al.* 2001, Neiman *et al.* 2013b) inspired me to evaluate potential effects of lake of origin. These lake-level analyses are particularly interesting in light of evidence that the strength and type of selection for life history traits such as growth rate might vary across or within *P. antipodarum* populations (Jokela and Lively 1995, Krist *et al*. 2014). I evaluated my dataset for lake effects by using general linear models to determine whether the fixed factors of lake of origin and random factor of family (~genetic variation; nested within lake of origin) influenced growth rate, age at maturity, and final length. The sample sizes for diploid and tetraploid families were too small (four lakes with 2x families, five lakes with 4x families) for meaningful analysis, so I confined these analyses to the 76 triploid families from 14 lakes.

I addressed the question of whether sexual reproduction confers higher variance in fitness-related traits relative to asexuals by comparing the variance for each of the three life history traits that I measured in sexual and asexual *P. antipodarum* (following Becks and Agrawal 2011). I began by using a variance components analysis to estimate the variance of each trait for each of the 11 sexual and 92 asexual families that I included in my experiment. K-S analyses revealed that all three sets of family variances required transformation to meet the normal distribution requirement of parametric statistical analysis (final length: K-S statistic = 0.171, df = 83, $p < 0.0001$; growth rate: K-S statistic = 0.157, df = 79, $p = < 0.0001$; age at maturity: K-S statistic = 0.223, df = 85, $p < 0.0001$). After natural log transformations of these

data, the variances of age at maturity (K-S statistic = 0.087, df = 77, p = 0.200) and final length (K-S statistic = 0.094, df = 77, p = 0.091) were normally distributed. The growth rate variance data met the normality requirement following a cube root transformation (K-S statistic = 0.072, df = 79, p = 0.200).

I then used these transformed family variances as the dependent variables in univariate general linear models with reproductive mode as a fixed factor, and estimated 95% confidence intervals around the mean trait variance for sexual *vs*. asexual families with 1000 bootstrap replications. I used the same procedure to compare the mean family variances in the 76 asexual triploid families *vs*. the 16 tetraploid families (with ploidy as a fixed factor) as well as between triploid families with field collected *vs*. laboratory-reared founder source (with founder source as a fixed factor). The ploidy and founder source analyses allowed me to address whether ploidy level *per se* and/or the potential for adaptation to the laboratory environment in the G1s with laboratory-reared mothers, respectively, might affect the variance in expression of life history traits (Becks and Agrawal 2011). All statistical analyses (including power analyses, using the "opower" function) were conducted with IBM SPSS Statistics version 21.

Table 1. Characteristics of founding females and G1 offspring

Lake of Origin	Source	# Founding Females	# Founders that Reproduced	Males Added	# 2x Families	# 3x Families	# 4x Families
Alexandrina (shallow)	Field	20	16	Yes	3	10	0
Alexandrina (*Isoetes*)	Field	20	12	Yes	4	7	0
Clearwater	Lab	1	1	No	0	1	0
Clearwater	Lab	1	1	No	0	1	0
Grasmere	Field	20	14	Yes	1	12	0
Gunn	Lab	1	1	No	0	0	1
Gunn	Lab	1	1	No	0	0	1
Gunn	Lab	1	1	No	0	0	1
Gunn	Lab	1	1	No	0	0	1
Gunn	Lab	1	1	No	0	0	1
Gunn	Lab	1	1	No	0	1	0
Gunn	Lab	1	1	No	0	0	1
Gunn	Lab	1	1	No	0	0	1
Gunn	Lab	1	1	No	0	0	1
Gunn	Lab	1	1	No	0	1	0
Haupiri	Field	20	7	Yes	0	3	1
Heron	Field	20	19	Yes	0	18	1
Kaniere	Lab	1	1	No	0	1	0
Kaniere	Lab	1	1	No	0	1	0
Okareka	Lab	1	1	No	0	1	0
Okareka	Lab	1	1	No	0	1	0
Okareka	Lab	1	1	No	0	1	0
Okareka	Lab	1	1	No	0	1	0
Poerua	Lab	1	1	No	0	1	0
Poerua	Lab	1	1	No	0	1	0
Poerua	Lab	1	1	No	0	0	1
Poerua	Lab	1	1	No	0	0	1
Poerua	Lab	1	1	No	0	1	0
Poerua	Lab	1	1	No	0	1	0
Rotoiti	Lab	1	1	No	0	0	1
Rotoiti	Lab	1	1	No	0	0	1
Rotoiti	Lab	1	1	No	0	1	0
Rotoiti	Lab	1	1	No	0	0	1
Rotoiti	Lab	1	1	No	0	0	1
Rotoroa	Field	20	10	Yes	1	4	0
Selfe	Field	20	11	Yes	3	5	0
Taylor	Lab	1	1	No	0	1	0
Waikaremoana	Lab	1	1	No	0	1	0

RESULTS

Effects of ploidy and reproductive mode on life history traits

I did not detect a significant effect of ploidy level on growth rate (Fig. 1, Fig. 2, Table 2; Tukey's honestly significant difference: 2x-3x: $p = 0.441$; 2x-4x: $p = 0.064$; 3x-4x: $p = 0.140$). Because growth rate in triploids and tetraploids was statistically indistinguishable, I combined the triploids and tetraploids into one "asexual" category and then conducted a general linear model analysis identical to that used for the ploidy analysis except that I replaced the fixed factor of ploidy with the fixed factor of mode of reproduction (sexual *vs.* asexual). Although this analysis did not reveal a significant difference in growth rate between reproductive modes, the significant effect of family in both the ploidy level and reproductive mode analyses (Table 2) indicates the existence of substantial genetic variation for growth rate in natural populations of sexual and asexual *P. antipodarum*.

While there was not a significant main effect of ploidy level on age at maturity (growth rate residuals) (Table 2), Tukey's posthoc pairwise analyses revealed that diploids matured significantly more slowly than triploids ($p = 0.023$) but that triploid and tetraploid asexuals were statistically indistinguishable ($p = 0.636$) (Fig. 3; also see Fig. 4). Accordingly, I again combined triploids and tetraploids into an asexual category and used the same general linear model approach to evaluate whether sexuals and asexuals differed in age to maturity. This analysis revealed a marginally significant effect of reproductive mode and a significant effect of family on age at maturity, demonstrating genetic variation for this important life history trait (Table 2).

The growth rate-uncorrected age at maturity analyses did reveal a significant effect of ploidy level (Table 2), with diploids maturing at a significantly later age than both triploids and tetraploids (Tukey's honestly significant difference, $p < 0.0001$; Fig. 3). Because there was no

significant difference in age at maturity between triploids and tetraploids ($p = 0.101$), I again combined the triploids and tetraploids into an asexual category and used the same univariate general linear model approach as before to address the effect of reproductive mode on uncorrected age at maturity. Here, I detected a significant effect of reproductive mode (Table 2) on age to maturity, driven by the ~30% increase in the number of days it took sexuals to achieve reproductive maturity relative to asexuals (Fig. 3).

Although there was no significant main effect of ploidy level on final length (growth rate residuals) ($p = 0.077$; Table 2, Fig. 5; also see Fig. 6), tetraploids were significantly larger than triploids ($p = 0.026$). Diploids and triploids ($p = 0.791$) and diploids and tetraploids ($p = 0.099$) were statistically indistinguishable. Final length differed significantly across families (Table 2), again revealing genetic variation in *P. antipodarum* for an important life history trait.

Analysis outcomes using the uncorrected final length data were similar to the growth rate residual-corrected analyses of final length (Table 2), with the exception that diploid sexual female *P. antipodarum* (mean = 4.8028 +/- 0.611394) had significantly shorter shell lengths at reproductive maturity than their triploid counterparts (triploid mean = 5.03700 +/- 0.624977, $p = 0.033$; Fig. 5). There was not a significant difference in shell length between triploids and tetraploids (tetraploid mean = 4.9031 +/- 0.71510; $p = 0.073$), indicating that the significant differences in growth rate-corrected final length that I observed between triploids and tetraploids are not evident when the growth rate-shell length association is left intact. Diploids and tetraploids ($p = 0.596$) were also statistically indistinguishable (Fig. 5).

The intermediate ploidy level (triploid) *P. antipodarum* had the longest shells, suggesting that ploidy level variation *per se* does not account for the relatively short shell length of diploid sexual females. A reproductive mode-focused univariate general linear model analysis

comparing the final length of diploid sexuals to the pooled triploid and tetraploid asexuals indicated a significant effect of family and thus substantial genetic variation for final length, but no significant difference in final length between sexuals and asexuals (Table 2; also see Fig. 5).

This latter result is consistent with and extends to the species level a previous study finding no significant difference in shell length at reproductive maturity in sexual and asexual female *P. antipodarum* from lake Alexandrina (Jokela *et al.* 1997b). This result also suggests that the significant difference in final length between diploids and triploids that I detected is not simply attributable to reproductive mode. Instead, the significantly larger shell length of the triploid *vs*. diploid *P. antipodarum* could be linked to heritable size differences in *P. antipodarum* sampled from different lakes (see below). I also addressed this possibility by using the same general linear model structure as before to compare final size in the six sexual and 15 asexual Alexandrina families, the one lake for which I had enough sexual and asexual families to perform a meaningful comparison. There was no difference in final size between sexual and asexual females ($F_{(1, 36.686)} = 0.015$, $p = 0.902$), although the small effect size (sexual mean = 5.0817 mm and asexual mean = 5.0593 mm) and low power of this analysis (5.2%) means that this negative result must be viewed with caution. Nevertheless, the data considered together suggest that direct effects, if any, of ploidy and/or reproductive mode on size at reproductive maturity are weak (also see Jokela *et al.* 1997b) and that the significantly larger size of triploid *vs*. diploid *P. antipodarum* might indeed be linked to a lake effect.

The fact that female *P. antipodarum* of all three ploidy levels and both reproductive modes do not reproduce until reaching at least 4.0 mm in shell length combined with the markedly slower attainment of reproductive maturity in sexual *vs*. asexual females and the strong negative correlation between growth rate and age at maturity (see below) suggests that a

potential driver of these differences in rate of maturation is relatively rapid growth in asexual *vs.* sexual *P. antipodarum.* While these differences were not apparent in my comparisons of growth rate to 3.0 mm (Fig. 5), a general linear model evaluating how the fixed factor of reproductive mode influenced the dependent variable of days between reaching 3.0 mm in shell length and first reproduction (with the random factor of family nested within reproductive mode) indicated that asexual *P. antipodarum* take significantly fewer days (56% of days relative to sexuals) to reproduce after reaching 3.0 mm than their sexual counterparts ($F_{(1, 190.348)} = 15.271$, $p < 0.0001$; Fig. 7; also see Fig. 8). This result is consistent with a scenario where asexual *P. antipodarum* reproduce at an earlier age than sexual *P. antipodarum* because the former grow more rapidly than the latter. The significantly higher age at maturity of sexual *vs*. asexual *P. antipodarum* revealed by the general linear model analysis using uncorrected (*i.e.,* growth rate association not removed) age to maturity data compared to the only marginally significant outcome of the same analysis using growth rate-corrected age at maturity data further supports this conclusion (Fig. 7).

Another line of support for the possibility that a higher growth rate underlies the relatively early reproductive maturity of asexual *P. antipodarum* comes from a Pearson's correlation analysis revealing a significant negative correlation between growth rate and age at maturity ($R^2 = -0.622$, $p < 0.0001$) but no relationship between growth rate and final size ($R^2 = 0.098$, $p = 0.098$). The tight association between growth rate and age at maturity highlights the likelihood that growth rate is an important fitness-related trait in *P. antipodarum*.

Evaluating effects of lake of origin on life history variation in asexual P. antipodarum

I did not detect significant effects of lake of origin on any of my three focal life history traits (growth rate: $F_{(13, 61.597)} = 0.973$, $p = 0.488$; growth rate-corrected age at maturity: $F_{(13, 56.101)} = 0.692$, $p = 0.763$; growth rate-corrected final size: $F_{(13, 61.460)} = 1.691$, $p = 0.086$). The power of these analyses was moderately high (0.522-0.818), suggesting that I would have detected strong effects of lake of origin. There was a significant effect of family, and thus, genetic variation within lakes, for growth rate ($F_{(62, 184)} = 1.728$, $p = 0.003$) and final size ($F_{(57, 185)} = 1.414$, $p < 0.0001$), but not for age at maturity ($F_{(60, 164)} = 1.254$, $p = 0.133$), indicating low levels of genetic variation for age at maturity (after the effect of growth rate is removed) relative to the other two traits. The analysis of the uncorrected age at maturity data still detected no effect of lake ($F_{(13, 62.141)} = 1.582$, $p = 0.115$, observed power = 0.786) but did reveal a significant effect of family ($F_{(61, 208)} = 2.378$, $p < 0.0001$). This latter result suggests that while age at maturity is heritable, selection on this trait will likely impose correlated selection on growth rate, with the implication that these two traits cannot be selected independently.

Effects of reproductive mode, ploidy level, and founder source on life history trait variances

There were no significant differences in mean variance in any of the three life history traits between reproductive modes or between triploid and tetraploid asexuals (Table 3). These variance results suggest that sexual reproduction may not create a variance-related benefit of sex in *P. antipodarum*, at least for these traits and in a laboratory environment, though the low statistical power (< 0.384) of these analyses means that these variance results should be viewed with caution. The absence of obvious differences in variance between triploids and tetraploids also indicates that phenomena associated with extra genomes (*i.e.*, higher heterozygosity, mitotic

instability; reviewed in Comai 2005) do not necessarily influence offspring trait expression.

I did detect an effect of founder source on age at reproduction within triploids (Table 3), whereby families produced by laboratory-raised founders had significantly less variance than families produced by field-collected founders. This result could reflect the homogeneity of selection imposed by a laboratory *vs*. field environment, and hints that the trait values expressed in the laboratory might not always reflect those in natural populations (also see discussion).

Table 2. Summary of outcomes of univariate general linear models evaluating the effect of ploidy level and reproductive mode on life history traits. I pooled triploids and tetraploids for "reproductive mode" analyses contrasting the diploid sexuals with the triploid and tetraploid asexuals only if post-hoc Tukey's tests conducted as part of "ploidy" analyses showed that triploids and tetraploids were not statistically distinguishable ($p > 0.05$).

Trait	Effect	$F_{(df)}$	p
Growth rate	Ploidy	$1.347_{(2, 129.52)}$	0.264
	Family (ploidy)	$2.038_{(99, 263.00)}$	< 0.0001
	Reproductive mode	$0.305_{(1, 164.06)}$	0.581
	Family (reproductive mode)	$2.082_{(99, 264.00)}$	< 0.0001
Age at maturity	Ploidy	$2.182_{(2, 168.99)}$	0.116
	Family (ploidy)	$1.383_{(97, 222.00)}$	0.026
	Reproductive mode	$3.850_{(1, 245.68)}$	0.055
	Family (reproductive mode)	$1.377_{(98, 222.00)}$	0.027
	Ploidy*	$4.313_{(2, 139.40)}$	0.015
	Family (ploidy)*	$2.644_{(99, 280.00)}$	< 0.0001
	Reproductive mode*	$6.940_{(1, 177.00)}$	0.009
	Family (reproductive mode)*	$2.670_{(100, 280.00)}$	< 0.0001
Final length	Ploidy	$2.604_{(2, 143.27)}$	0.077
	Family (ploidy)	$1.774_{(93, 208.00)}$	< 0.0001
	Ploidy*	$0.067_{(2, 109.13)}$	0.510
	Family (ploidy)*	$4.647_{(94, 260.00)}$	< 0.0001
	Reproductive mode*	$0.956_{(1, 134.62)}$	0.330
	Family (reproductive mode)*	$4.647_{(94, 260.00)}$	< 0.0001

*Denotes data uncorrected for significant associations with growth rate

Table 3. The outcome of univariate general linear models comparing family variances in growth rate, age at maturity, and final length between sexual (Sex) and asexual (Asex) *P. antipodarum*, triploid and tetraploid asexual *P. antipodarum*, and laboratory-raised vs. field-collected founder families. Δ = Variance $_{Sex}$ − Variance $_{Asex}$, Variance $_{Triploid}$ − Variance $_{Tetraploid}$, or Variance $_{Laboratory}$ − Variance $_{Field}$.

		Mean Variance*	95% CI	Δ	95% CI for Δ	*p*-value for Δ	Observed power
Growth rate	Sex	0.022	0.015 − 0.030	-0.005	-0.003 − 0.013	0.247	0.210
	Asex	0.027	0.024 − 0.029				
	Triploid	0.021	0.017 − 0.025	0.000	-0.007 − 0.007	0.894	0.052
	Tetraploid	0.020	0.015 − 0.026				
	Lab Founders	0.021	0.015 − 0.020	0.001	-0.007 - 0.009	0.763	0.06
	Field Founders	0.020	0.016 − 0.024				
Age at maturity	Sex	8.048	6.727 − 9.228	0.340	-1.550 − 1.069	0.613	0.093
	Asex	7.707	7.390 − 8.080				
	Triploid	7.849	7.452 − 8.239	0.695	-1.577 − 0.106	0.118	0.384
	Tetraploid	7.154	6.261 − 7.832				
	Lab Founders	7.085	6.516 − 7.638	-1.108	-1.914 − -0.303	0.008	0.773
	Field Founders	8.193	7.714 − 8.616				
Final length	Sex	-2.240	-1.035 − 0.651	-0.192	-1.081 − 1.466	0.759	0.073
	Asex	-2.048	-2.290 − -1.810				
	Triploid	-2.060	-2.345 − -1.780	0.019	0.647 − 0.601	0.963	0.050
	Tetraploid	-2.079	-2.679 − -1.513				
	Lab Founders	-2.09	-2.629 − -1.560	-0.06	-0.696 − 0.577	0.859	0.054
	Field Founders	-2.035	-2.351 − - 1.735				

*Values are calculated from the transformed variances

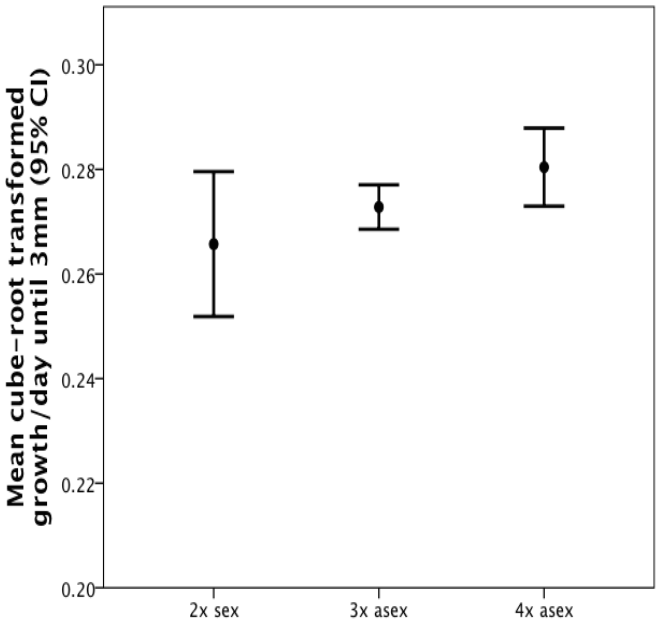

Figure 1. Mean growth rate until 3.0 mm in shell length across ploidy levels. I show untransformed data here in order to facilitate interpretability; the comparisons involving the cube root-transformed data are qualitatively identical

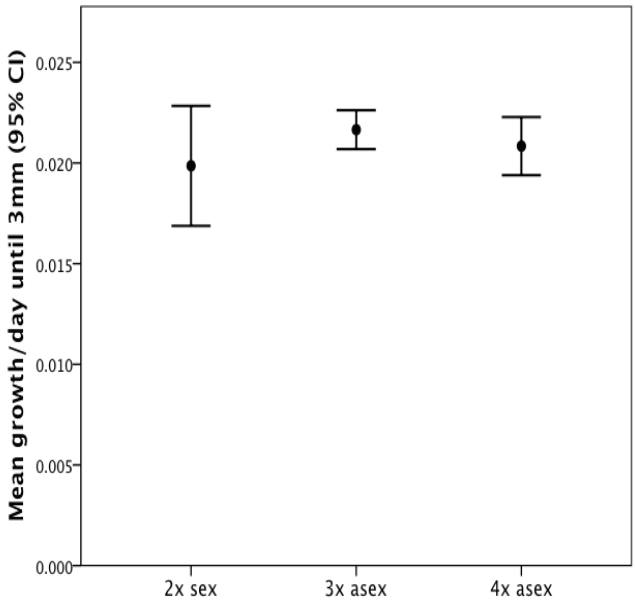

Figure 2. Mean growth rate until 3.0 mm in shell length across ploidy levels using the transformed data. I rescaled the y-axis in order to facilitate visual comparisons.

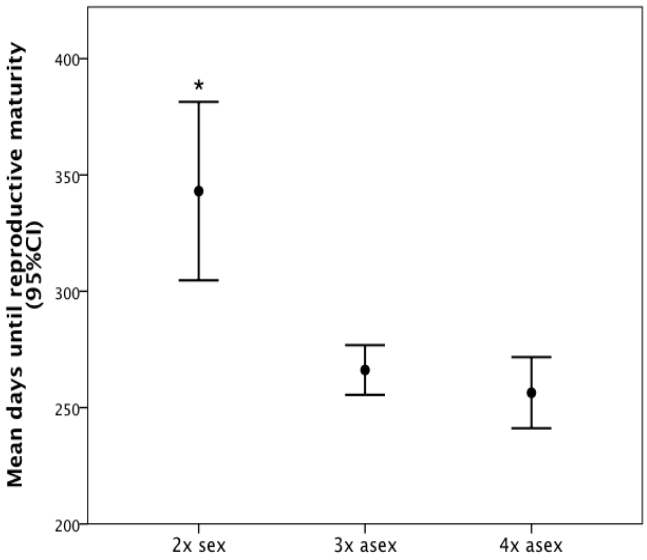

Figure 3. Mean days until reproductive maturity across ploidy levels. I show uncorrected and untransformed data here in order to facilitate interpretability; the comparisons involving the natural log-transformed residual data are qualitatively similar, although the removal of the effect of growth rate rendered the overall effect of reproductive mode and the diploid-tetraploid comparisons non-significant

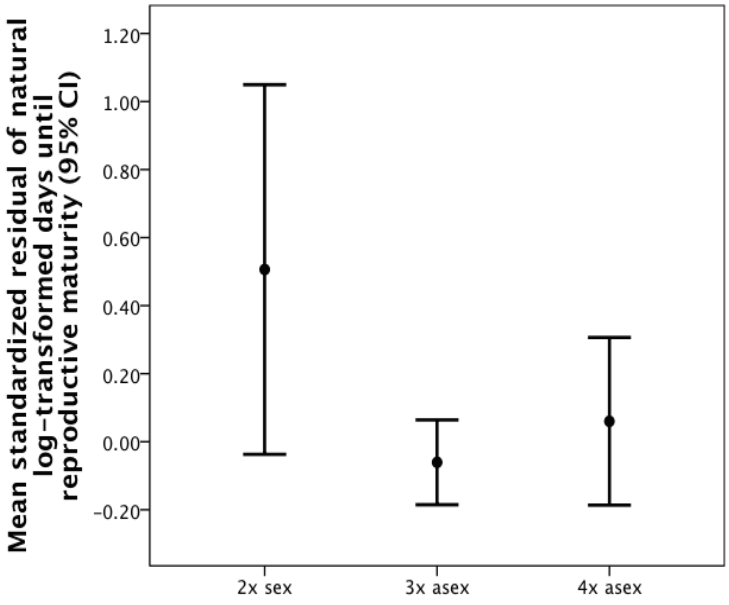

Figure 4. Mean days until reproductive maturity across ploidy levels using the growth rate-corrected transformed data

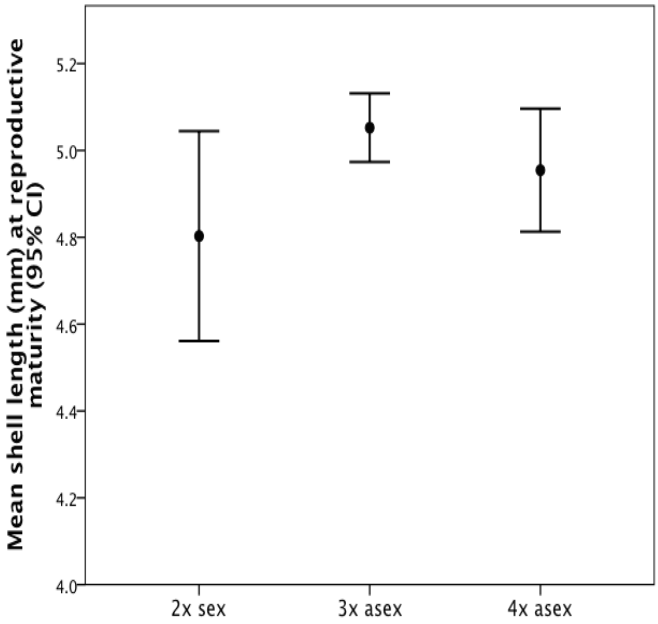

Figure 5. Mean shell length at reproductive maturity across ploidy levels. I show uncorrected and untransformed data here in order to facilitate interpretability; the comparisons involving the natural log-transformed residual data are similar in that there is no significant main effect of ploidy. These comparisons differ in that tetraploids have significantly longer final lengths here, but are significantly longer than the triploids (and indistinguishable from the diploids) in the transformed residual dataset

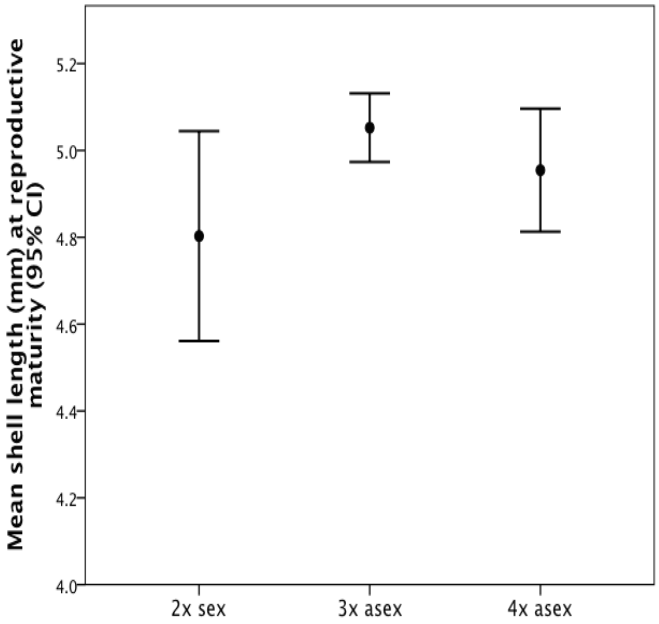

Figure 6. Mean shell length at reproductive maturity using the growth rate-corrected transformed data

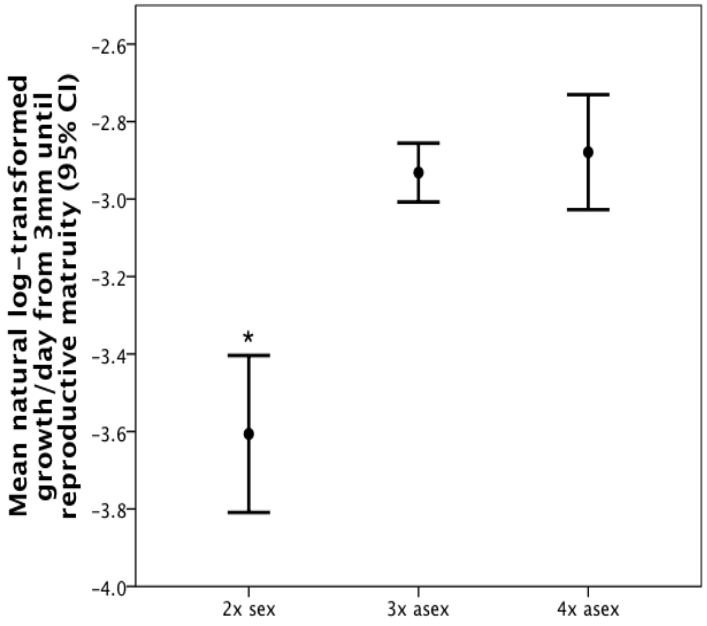

Figure 7. Mean growth per day from 3.0 mm in length until reproductive maturity. I show uncorrected and untransformed data here in order to facilitate interpretability; the comparisons involving the natural log-transformed residual data are qualitatively identical

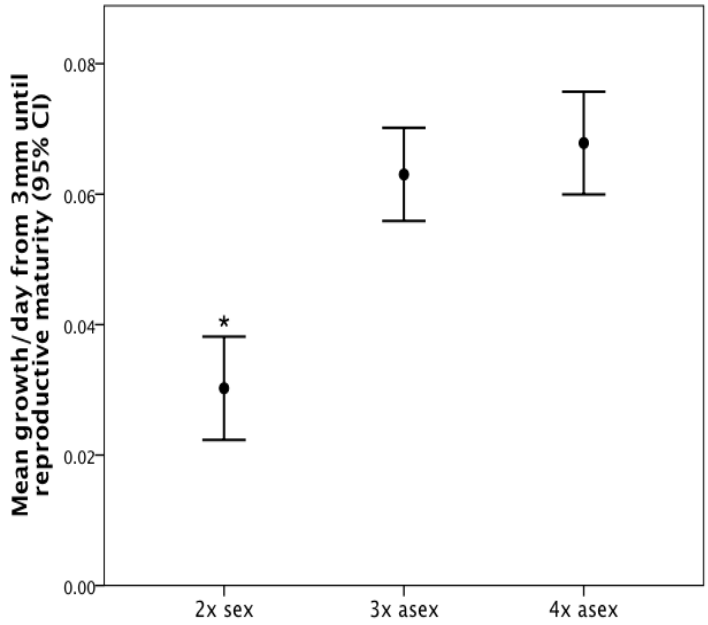

Figure 8. Mean growth per day from 3.0 mm in length until reproductive maturity using the transformed data. I rescaled the y-axis in order to facilitate visual comparisons

DISCUSSION

I used a common garden approach to evaluate whether life history traits differ across ploidy level, reproductive mode, and lake of origin in *Potamopyrgus antipodarum*, an important natural model system for the study of ploidy variation, the evolution of sex, and host-parasite coevolution. My study revealed extensive genetic variation for all three life history traits that I measured (growth rate, age at maturity, final length) and suggested that reproductive mode was likely to be a much more important contributor to life history trait expression than ploidy level or lake of origin. In particular, I found that diploid sexual *P. antipodarum* grow 44% slower and take about 79 days (30%) longer to reach reproductive maturity than their polyploid asexual counterparts. The consistent absence of differences in any life history trait values between triploid and tetraploid asexual *P. antipodarum*, with the exception of growth rate-corrected final length, indicates that effects of ploidy level are not likely to explain the significantly longer time to maturity in sexual diploid *P. antipodarum*.

My results generally depart from other studies that have evaluated the life history consequences of ploidy elevation, which often find that polyploids develop more slowly (e.g., Lowcock 1994, von Well and Fossey 1998, Elìášová and Münzbergová 2014) and have larger bodies (Otto and Whitton 2000, Gregory and Mable 2005) than their diploid counterparts. Most of these previous studies were conducted on hybrid polyploids and/or plant (usually hybrid) polyploids (Mable *et al*. 2011), raising the possibility that non-hybrid polyploid animals experience different (and relatively minor) consequences of ploidy elevation relative to their hybrid and/or plant counterparts. This suggestion finds indirect support from the multiple studies (nearly all in plants) that have demonstrated that allopolyploidy has a much larger effect on gene expression than autopolyploidy, implicating hybridization rather than ploidy elevation *per se* as

the causal factor in gene expression changes following a transition to polyploidy (reviewed in Neiman *et al*. 2013a).

My results instead suggest that reproductive mode might directly influence life history trait expression, though I cannot formally rule out the alternative explanation that the transition from diploid to triploid confers substantially more consequences than the triploid to tetraploid transition. An important potential role of growth rate as a primary driver of the life history differences that I detected between sexual and asexual *P. antipodarum* is revealed by the combination of an apparent threshold size for female reproductive maturity (~4.0 mm in shell length; also see Winterbourn 1970, McKenzie *et al*. 2013), the negative association between growth rate and age at reproduction, and the significantly longer time to reproductive maturity of sexual diploid female *P. antipodarum*.

Because a higher growth rate translates into likely fitness advantages (earlier reproduction) for female *P. antipodarum* (also see Tibbets *et al.* 2010), my results raise the questions of why there exists substantial genetic variation for this trait in *P. antipodarum* and why sexual *P. antipodarum* grow and mature relatively slowly. With respect to the maintenance of genetic variation for growth rate, the well-documented dependence of growth rate in *P. antipodarum* on environmental variables like food quality (Neiman *et al*. 2013b, Krist *et al.* 2014), food quantity (Neiman *et al*. 2013c), temperature (Dybdahl and Kane 2005), and population density (Neiman *et al*. 2013c, Zachar and Neiman 2013) suggest that at least some of the genetic variation for growth rate observed in benign laboratory conditions may be suppressed or expressed differently in the more heterogeneous natural populations. Indirect support for this possibility is provided by Neiman *et al.* (2013b), who found that while growth rate advantages are experienced by tetraploid *P. antipodarum* fed a high-phosphorus diet (and a diet higher in

phosphorus than the snails in the present study received), these growth rate advantages disappear under relatively low-P conditions. The results of Neiman *et al*. (2013b) emphasize the possibility that I may have detected life history disadvantages associated with polyploidy had I raised the snails in this experiment in harsher (e.g., low food quantity or quality) conditions. More broadly, rigorous evaluation of the extent to which environmental variation might influence growth rate and other important life history traits in natural *P. antipodarum* populations will ultimately require quantification of these traits in the field.

Another possibility is that more rapid growth and/or earlier maturation confer other, as yet unmeasured, costs (Blankenhorn 2000, Mangle and Stamps 2001). Such tradeoffs have often been documented in other taxa, and include costs such as an increased rate of developmental deformities (Sibly and Calow 1986), decreased maintenance and repair of proteins and DNA (Roff 1984), and decreased immune (Arendt 1997) and cellular functions (Ricklefs *et al*. 1998). Under the assumption that these or other costs associated with more rapid growth and maturation might plausibly affect survivorship, I used a Fisher's Exact Test to compare the proportion of sexual *vs*. asexual and diploid *vs*. triploid *vs*. tetraploid *P. antipodarum* that survived to reproductive maturity. I found that the sexuals were 17% more likely to die prior to reproduction than the asexuals as a whole ($p = 0.0028$; Fig. 9). Comparisons of mortality between the diploid sexuals and the triploid ($p = 0.0034$) and tetraploid ($p = 0.0110$) asexuals were qualitatively identical (Fig. 10). By contrast, there was no detectable difference in the proportion of triploid *vs*. tetraploid asexuals that died prior to reproduction ($p = 1.000$). Altogether, these analyses provide no evidence either for a growth rate-mortality tradeoff or obvious effects of elevated ploidy on mortality in my experiment (Fig. 10) and emphasize the conclusion that sexuals appear to suffer life history disadvantages.

Life history trait variation can also generate population-level effects that can influence the maintenance of individual-level variation for these traits (Pfister 1998, Beckerman *et al.* 2002). Potential connections between individual life history trait expression and population dynamics have already been illustrated in *P. antipodarum* by Pedersen *et al*. (2009), who showed that a change in fecundity (itself positively associated with shell length in *P. antipodarum*) will have much less of an impact on population growth than proportional changes in other life history traits such as individual growth rate, time to first reproduction, or survival. The results reported by Pederson *et al.* (2009) suggest that feedbacks between individual trait values and population dynamics in *P. antipodarum* are thus more likely to help explain the maintenance of genetic variation for shell length but less relevant to understanding the maintenance of genetic variation for growth rate and age at maturity.

Another potential non-mutually exclusive explanation for why sexual *P. antipodarum* grow and mature more slowly than their asexual counterparts may be provided by the higher per-unit mass RNA content (Neiman *et al*. 2009) and tissue regeneration rate (Krois *et al*. 2013) of asexual *vs*. sexual *P. antipodarum*. Both of these results suggest that asexual *P. antipodarum* may realize tissue- and individual-level growth advantages connected to higher per-organism gene expression levels (Neiman *et al*. 2013a). The extent to which this type of mechanism might help explain the life history differences between sexual and asexual *P. antipodarum* will require in-depth characterization of gene expression levels in sexuals and asexuals and evaluation of how gene expression differences (if any) translate into differences in life history trait expression.

Selection can act very efficiently to promote high-quality genotypes when asexual populations harbor high genetic diversity (Wright 1997; reviewed in Neiman and Linksvayer 2006). This connection between the efficacy of selection and asexual diversity combined with

the notably high genetic diversity of New Zealand asexual *P. antipodarum* (Jokela *et al*. 2003, Paczesniak *et al*. 2013) thus raises the possibility of yet another non-mutually exclusive explanation for the higher performance of asexual *P. antipodarum*: asexual *P. antipodarum* might grow more rapidly and reproduce earlier than their sexual counterparts because genotypes that contribute to rapid growth and earlier reproduction are not broken up by recombination. Some testable predictions stem from this hypothesis, including the expectations that growth-related traits in *P. antipodarum* should be affected by multiple loci that are not physically linked (Koehn *et al*. 1988) and that there should be less variation in life history traits within asexual sibling groups than in sexual sibling groups.

The latter prediction was not upheld in my comparisons of mean trait variance between sexual and asexual families, though the interpretation of this negative result is complicated by the fact that sexual and asexual *P. antipodarum* are sometimes (but not always) genetically distinct (Neiman and Lively 2004, Paczesniak *et al*. 2013) and the possibility that the offspring produced in my experiment by a single sexual female did not have the same father (also see Soper *et al*. 2012). Multiple paternity would tend to result in a bias towards inflated variance in sexual families, however, indicating that this particular factor is not likely to explain the absence of significant differences in trait variation in sexually *vs*. asexually reproduced *P. antipodarum.*

Altogether, my survey of life history variation across ploidy levels and reproductive modes in *P. antipodarum* indicates that higher ploidy does not confer obvious life history costs. More broadly, these results suggest that the effects of ploidy elevation, if any, are either weak or non-linear (e.g., the transition from diploid to triploid affects phenotype substantially more than the transition from triploid to tetraploid) under the benign laboratory conditions used in this experiment. In the absence of direct evidence for individual- or population-level costs or

tradeoffs associated with more rapid growth or reproduction, the notably slower rate of maturation of sexual *vs*. asexual *P. antipodarum* indicates that sexual females experience even more than the two-fold cost of sex already documented for sexual *P. antipodarum* (Jokela *et al.* 1997b). My study serves as a qualitative advance relative to earlier work on *P. antipodarum* by including a focus on effects of both ploidy and reproductive mode, the inclusion of multiple natural lake populations, and the evaluation of several distinct life history traits. These results are thus likely to extend to the species as a whole, indicating that there exist major benefits associated with sexual reproduction that allow sexual diploid *P. antipodarum* to overcome what appear to be substantial life history disadvantages and persist in some natural populations. Evidence that parasite-mediated negative frequency-dependent selection is likely to help favor sexual *P. antipodarum* in at least some New Zealand populations (e.g., Lively 1987, Jokela *et al*. 2009) is consistent with this conclusion.

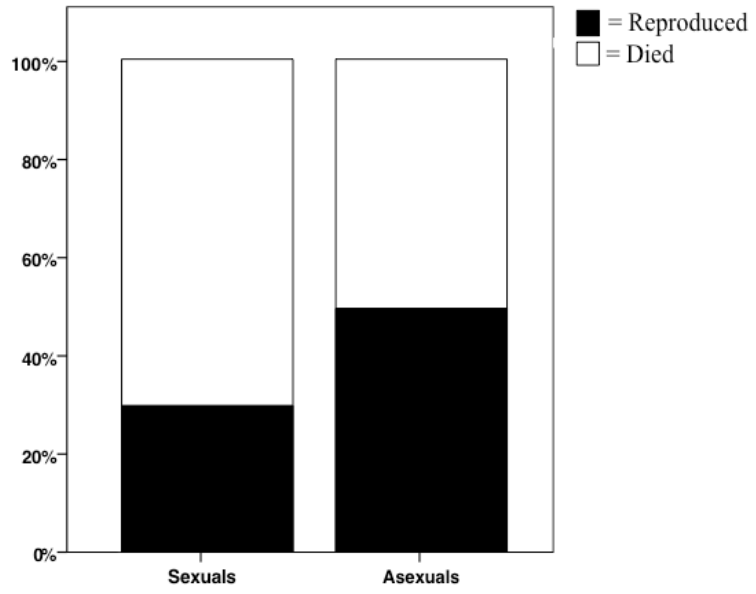

Figure 9. Proportion of sexual and asexuals that reproduced and the proportion of sexuals and asexuals that died prior to reproduction. A Fisher's exact test revealed that a significantly higher proportion of sexuals died prior to reproduction than asexuals (p = 0.0028).

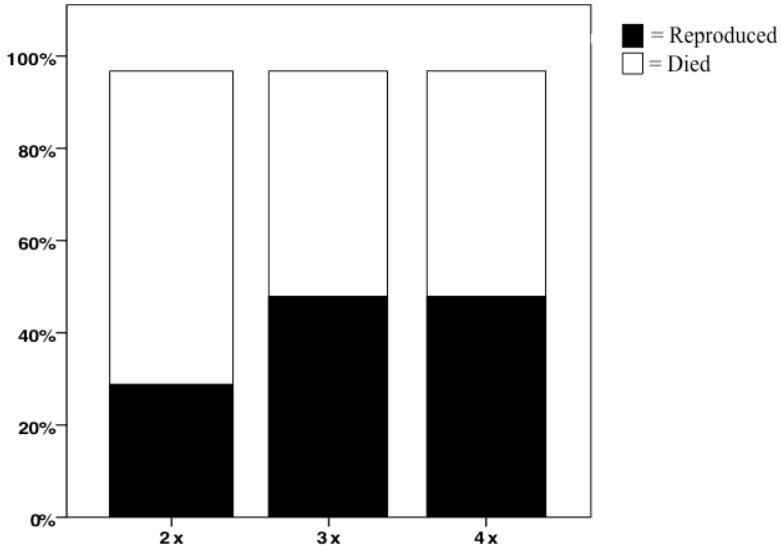

Figure 10. Proportion of 2x, 3x, and 4x snails that reproduced and the proportion of 2x, 3x, and 4x snails that died prior to reproduction. Fisher's exact tests revealed that a significantly higher proportion of sexuals died prior to reproduction than triploid asexuals (p = 0.0034) and relative to tetraploid asexuals (p = 0.0110). There was no significant difference in the proportion of 3x *vs.* 4x snails that died prior to reproduction (p = 1.0000).

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