doi:10.1111/evo.12026

SENSITIVITY TO PHOSPHORUS LIMITATION INCREASES WITH PLOIDY LEVEL IN A NEW ZEALAND SNAIL

Maurine Neiman,[1,2] Adam D. Kay,[3] and Amy C. Krist[4]

[1]*Department of Biology, University of Iowa, Iowa City, Iowa*

[2]*E-mail: maurine-neiman@uiowa.edu*

[3]*Department of Biology, University of St. Thomas, St. Paul, Minnesota*

[4]*Department of Zoology and Physiology & Program in Ecology, University of Wyoming, Laramie, Wyoming*

Received September 2, 2012
Accepted November 27, 2012
Data Archived: Dryad doi:10.5061/dryad.ct601

Evolutionary and ecological factors that explain natural variation in ploidy level remain poorly understood. One intriguing possibility is that nutrient costs associated with higher per-cell nucleic acid content could differentially influence the fitness of different ploidy levels. Here, we test this hypothesis by determining whether access to phosphorus (P), a main component of nucleic acids, differentially affects growth rate in asexual freshwater snails (*Potamopyrgus antipodarum*) that differ in ploidy. As expected if larger genomes generate higher dietary P requirements, tetraploid *P. antipodarum* experienced a more than twofold greater reduction in growth rate in low-P versus high-P conditions relative to triploids. Mirroring these results, tetraploid *P. antipodarum* also had a significant reduction in body P content under low P relative to high P, whereas triploid body P content was unaffected. Taken together, these results set the stage for the possibility that P availability could influence the distribution and relative frequency of *P. antipodarum* of different ploidy levels. These findings could be applicable to many other animal taxa featuring ploidy-level variation, which includes many mixed sexual/asexual taxa.

KEY WORDS: **Ecological stoichiometry, growth rate, phosphorus, polyploidy, *Potamopyrgus antipodarum*, sexual reproduction.**

There is remarkable variation in eukaryotic genome size and structure (Gregory 2005; Lynch 2007). For example, the largest genomes contain at least 40,000× more DNA than the smallest (Gregory 2012), and eukaryotes can range from haploid (e.g., Weeks et al. 2001) to at least 38-ploid (Hair and Beuzenberg 1961; Bennett and Leitch 2010). Characterization of the evolutionary and ecological mechanisms maintaining this variation will provide novel and broadly important insights into how genome structure and size influence evolutionary processes and are affected by ecological context (e.g., Leitch and Leitch 2008; King et al. 2012).

Central to understanding the maintenance of ploidy-level variation is identifying ways in which polyploid phenotypes are likely to differ from diploid counterparts. This question has been of interest to biologists for decades, even drawing the attention of Albert Einstein (Fankhauser 1972). The costs and benefits of polyploidy have been the subject of many recent comprehensive reviews (e.g., Comai 2005; Otto 2007; Parisod et al. 2010; Mable et al. 2011; Albertin and Marullo 2012), so we here provide only a brief overview. In particular, polyploidy is often viewed as a positive trait either because of its direct effects on fitness-enhancing phenotypes (e.g., Ramsey 2011; reviewed in te Beest et al. 2012) or because it increases the raw material available for evolutionary innovation (reviewed in Otto and Whitton 2000; Parisod et al. 2010; Albertin and Marullo 2012; te Beest et al. 2012). However, polyploidy may also confer costs (reviewed in Comai 2005; Otto 2007). Examples of such costs include an increase in the equilibrium load of harmful mutations (Otto and Whitton 2000;

EVOLUTION INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

Otto and Gerstein 2009) and decreased rate of development (e.g., Fankhauser 1945; Cavalier-Smith 1978; Levin 1983).

Another potential cost of polyploidy may arise if the presence of extra genome copies influences nutrient demands because of increased allocation to nucleic acids (Lewis 1985; Hessen et al. 2008, 2010; Neiman et al. 2009, 2013; Van Geest et al. 2010). Because nucleic acids contain more phosphorus (P; approximately 9% dry mass) than other major biomolecules and can comprise a large fraction of organismal dry mass (Sterner and Elser 2002), allocation to nucleic acids accounts for much of the variation in whole-organism P concentration in a wide range of taxa (Elser et al. 2003). The high P content of nucleic acids has been predicted to result in positive relationships among ploidy, nucleic acid content, and dietary P in organisms ranging from microbial eukaryotes (Lewis 1985) to angiosperms (Leitch and Bennett 2004) to animals (Neiman et al. 2009, 2013). Consequently, nutrient costs should increase sensitivity to environmental P limitation in polyploid organisms. This hypothesis may be particularly applicable to invertebrate animals because nucleic acids comprise an especially high fraction of whole-organism P content (Sterner and Elser 2002).

No explicit tests of this hypothesis exist, although a few studies support some of the main assumptions. The one study of which we are aware that compared nucleic acid and phosphorus content between organisms differing in ploidy level found the expected approximately 50% increase in bodily nucleic acid and P content in triploid versus diploid New Zealand freshwater snails (*Potamopyrgus antipodarum*; Neiman et al. 2009). A few studies have provided evidence that constraints imposed by P limitation might influence the evolution of ploidy level or genome size in animals (e.g., Hessen et al. 2008; reviewed in Hessen et al. 2010). However, these studies have involved comparisons between allopatric and distantly related taxa that differ in many ways besides ploidy level, which ultimately permits only indirect inferences into the extent to which sensitivity to limited P is linked to ploidy level.

Here we report the most direct test to date of whether higher ploidy increases sensitivity to dietary P limitation in closely related and sympatric animals differing only in ploidy level. Our study species, *P. antipodarum*, a New Zealand freshwater snail, possesses several unusual traits that make it ideally suited to evaluate this hypothesis. First, the frequent coexistence of asexual triploid *P. antipodarum* with asexual *P. antipodarum* that have genome sizes exceeding triploidy (herein "tetraploid"; Neiman et al. 2011, 2012) allows for direct and powerful comparisons between closely related, sympatric, and phenotypically similar organisms differing in ploidy level. Second, individual growth rate and reproduction in triploid *P. antipodarum* increases with the P content of their food, indicating that polyploid *P. antipodarum* are sensitive to P limitation (Tibbets et al. 2010). Third, the first known survey of the relationship of ploidy level to body composition in animals demonstrated that mass-specific DNA, RNA, and P content generally increase with ploidy in *P. antipodarum* (Neiman et al. 2009).

For this study, we used triploid and tetraploid *P. antipodarum* to test whether ploidy affects individual growth rate (a main fitness correlate, Arendt 1997) under dietary P deficiency. Specifically, we predicted that a decrease in dietary P availability should result in a greater reduction in individual growth in tetraploids than triploids. Such growth rate responses would likely reflect fitness differences because *P. antipodarum* females that grow more slowly reach reproductive maturity later (Tibbets et al. 2010) and reproductive maturity and fecundity in *P. antipodarum* are positively linked to female size (Winterbourn 1970b; Tibbets et al. 2010). If polyploidy increases sensitivity to P limitation, it would suggest that competitive outcomes between individuals or subpopulations differing in ploidy could be mediated by environmental P availability.

Materials and Methods

We added one randomly sampled juvenile individual ($\sim$ 1.0 to 2.5 mm in length from shell aperture to tip) from each of 20 triploid *P. antipodarum* lineages and from each of 5 lineages of the rarer and recently discovered tetraploids (Neiman et al. 2011), to each of 24 2.4-L aquaria, for a total of 600 juvenile snails. Aquaria thus represented a blocking factor and will from this point on be referred to as "block." Hence, each snail within each block was a replicate for its lineage, and each lineage was a replicate within a ploidy level (triploid or tetraploid). We used nail polish to mark each individual within a block with a lineage-specific mark. All lineages were originally founded by a single asexual female collected from various populations in their native range in New Zealand or from invasive populations in Europe or North America. Descendants of these females were subsequently maintained in laboratory conditions; all individuals that descended from one of these females constitute a lineage. The ploidy of each of these 25 independently derived lineages was determined previously by flow cytometry (Neiman et al. 2011, 2012). Aquaria received continuously recirculating filtered well water and were kept in a 22°C to 24°C room on a 12 hour : 12 hour light/dark cycle.

In natural conditions, *P. antipodarum* consume green algae (e.g., Hicks 1997) and detritus (e.g., James et al. 2000). We used the green alga *Scenedesmus acutus* for our food treatments because we can easily manipulate its P content, have successfully reared *P. antipodarum* on *S. acutus*, and have demonstrated P limitation in *P. antipodarum* fed low-P versus high-P *S. acutus* (Tibbets et al. 2010). For this experiment, we reared snails on low P (C : P $\sim$ 1220; standard deviations (SD) for %C = 2.00, %P = 0.03) or high P (C : P $\sim$ 153; SD for %C = 1.27, %P = 0.19) *S. acutus* algae (12 replicates / diet). Diet molar C : P ratios were

above (low P) or below (high P) the threshold elemental ratio (Frost et al. 2006) for P limitation, estimated at C : P $\sim$ 270 for *P. antipodarum* (Tibbets et al. 2010). We added 0.0004g dried algae (resuspended in 30 ml well water) per snail three times per week; this amount of food is equivalent to an *ad libitum* food treatment under these conditions (high-food level in Krist et al. 2004). After adding algae, we turned off water flow for 15 hours to allow settling and minimize loss through outflow. We moved all the snails within each aquarium to a clean aquarium every 7 days.

We measured shell length (aperture to tip) at the beginning of the experiment, at day 18, and at day 36. At day 36, 35 individuals ($\sim$6.1% of all 570 snails alive at the end of the experiment) possessed embryos, indicating reproductive maturity. Because growth in *P. antipodarum* slows markedly near reproductive maturity (Winterbourn 1970b), we only used the initial 18-day period for estimating growth. At the end of the experiment we removed head tissue (for flow cytometry; data not included), snap froze the remaining body tissue, and then stored the tissue samples at -80°C.

We used these tissue samples to measure snail body P content to assess potential mechanisms for differential growth responses to P limitation. Following the methods used in Neiman et al. (2009), we used ascorbate-molybdate colorimetry to measure body P content for 242 *P. antipodarum* from the growth rate experiment. These 242 individuals represented all the snails from six blocks and included all but two of the 20 triploid lineages (76 individuals from the low-P treatment and 98 individuals from the high-P treatment) and all 5 tetraploid lineages (23 individuals from the low-P treatment and 29 individuals from the high-P treatment). Percent P recovery (111%–122%) was determined by comparison to bovine muscle standard (NIST 8414); P content of *P. antipodarum* was adjusted accordingly.

STATISTICAL ANALYSES

All statistical analyses were conducted with IBM SPSS (v. 19). We estimated specific growth rate as ln(length at 18 days/length at 0 days) / 18 days for each individual snail (Sterner and Elser 2002). Because there was a significant negative relationship between initial length and specific growth rate (linear regression, $r = -0.25$, $F_{1,583} = 39.63$, $P < 0.001$), we saved the residuals from this regression to provide a size-corrected estimate of growth rate. We then used univariate analysis of variance (ANOVA) to determine whether the fixed factors of ploidy level and phosphorus treatments alone or in interaction affected growth rate. To control for lineage effects, we nested the random factor of lineage within ploidy level. We also nested the random factor of block within food treatment. We addressed whether triploids and tetraploids were affected differently by the diet treatments by using univariate ANOVA (with the same basic model structure as above) to conduct post hoc comparisons within ploidy and treatment levels.

Table 1. Results of a univariate ANOVA evaluating the effects of the fixed factors of the ploidy and diet treatments and the random effects of lineage and block on specific growth rate (initial length residuals). Snail lineage was nested within ploidy level and blocks were nested within treatment.

Source	df (error)	MS (error)	F	P
Ploidy	1(22.98)	1.49(7.97)	0.19	0.670
Treatment	1(29.99)	22.38(1.53)	14.65	0.001
Treatment $*$ Ploidy	1(536)	3.95(0.62)	6.41	0.012
Lineage (Ploidy)	23(536)	7.93(0.62)	12.86	< 0.001
Block (Treatment)	22(536)	2.03(0.62)	3.3	< 0.001

Because growth in female *P. antipodarum* slows or stops at reproductive maturity (Winterbourn 1970b) and because P content is expected to vary with growth rate (Elser et al. 2003), we excluded reproductive individuals ($N = 16$) from the analysis of body P content. To control for the effects of body size, we used residuals from the log–log regression of individual body P mass and individual body biomass. We then used these residuals as the dependent variable, ploidy level and diet treatment as fixed main factors and in interaction, and block (nested within treatment), analysis date, and lineage (nested within ploidy level) as random factors in a univariate ANOVA. As for the growth rate experiment, we then used univariate ANOVA to conduct post hoc comparisons within ploidy and treatment levels to evaluate whether P content in triploids versus tetraploids were differentially affected by our diet treatments.

Results

All but 15 of the 600 experimental *P. antipodarum* (97.5%) survived for at least 18 days. The mean individual shell length growth per day (high P: 0.055 $\pm$ 0.01 mm/day SD; low P: 0.049 $\pm$ 0.009 mm/day) was similar to other studies that measured growth rates in juvenile *P. antipodarum* fed a variety of diets (Dorgelo et al. 1995; Broekhuizen et al. 2001; Dorgelo and Leonards 2001). The similarity in growth rates between our experiment and other studies suggests that the growth of the *P. antipodarum* in our experiment is a reasonable approximation of individual growth rate under adequate versus limited P.

The low-P diet had a marked negative effect on *P. antipodarum* specific growth rate (Table 1, Fig. 1), such that individuals in the low-P treatment grew approximately 14.5% less than individuals in the high-P treatment. A marked reduction in specific growth rate under low P versus high P was also apparent within both triploids (univariate ANOVA: $F_{1,22.02} = 6.15$, $P = 0.021$) and tetraploids ($F_{1,22.08} = 14.35$, $P = 0.001$). Overall, these results indicate that we successfully imposed P limitation in the low-P treatment for both triploids and tetraploids.

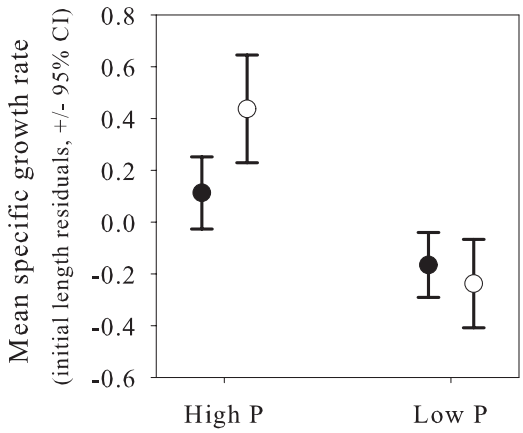

Figure 1. Relationship between initial length-corrected specific growth rate and diet P content for triploid (filled circles) and tetraploid (open circles) *Potamopyrgus antipodarum*. There was a significant positive effect of the high-P diet, demonstrating P limitation. We also detected a significant diet by ploidy interaction, which was caused by the much more severe reduction in specific growth rate for tetraploids in the low-P treatment relative to their growth in the high P treatment compared to triploid growth rate in high-P versus low-P conditions.

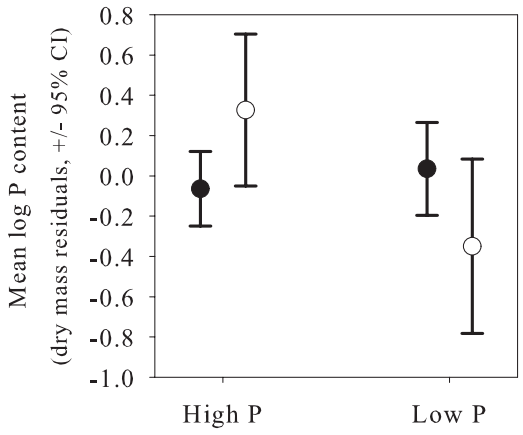

Figure 2. Relationship between snail P content (log mass residuals; y-axis) and dietary P content for triploid (filled circles) and tetraploid (open circles) *Potamopyrgus antipodarum*. There was a significant interaction between diet P content and ploidy level caused by the significant reduction in bodily P content of tetraploids but not triploids under low-P conditions.

We detected a significant ploidy-by-diet interaction for specific growth rate (Table 1, Fig. 1), which was driven by a reduction in growth rate in low-P versus high-P conditions that was substantially more severe for tetraploids (mean approximately 29% decrease in specific growth rate) than it was for triploids (mean approximately 11% decrease in specific growth rate). Specific growth rate was also affected by lineage (nested within ploidy; Table 1, Fig. S1) and block (nested within treatment; Table 1).

Table 2. Results of a univariate ANOVA evaluating the effects of the fixed factors of the ploidy and diet treatments and the random effects of lineage, block, and P analysis date on bodily P content (dry mass residuals). Snail lineage was nested within ploidy level and blocks were nested within treatment.

Source	df (error)	MS (error)	*F*	*P*
Ploidy	1(20.37)	0.36 (1.06)	0.34	0.569
Treatment	1(39.85)	1.57 (1.23)	1.27	0.266
Treatment ∗ Ploidy	1(185)	3.76 (0.92)	4.08	0.045
Lineage (Ploidy)	21(185)	1.06 (0.92)	1.15	0.302
Block (Treatment)	14(185)	1.62 (0.92)	1.76	0.047
P analysis date	2(185)	2.95 (0.92)	3.2	0.043

We detected a significant ploidy-by-diet interaction for *P. antipodarum* P content (Fig. 2, Table 2). Comparisons of P content within ploidy levels revealed that these effects of P treatments on P content were much more severe for tetraploids than for triploids. These results mirrored the outcome of the growth rate comparisons: although triploid P content was unaffected by P treatment ($F_{1,34.92} = 0.17$, $P = 0.685$), tetraploids experienced a marked reduction in body P content under low-P conditions ($F_{1,15.38} = 6.35$, $P = 0.023$). There was no significant effect of lineage (nested within ploidy; Table 2) on body P content, but there were significant effects of date of analysis (Table 2) and block (nested within treatment; Table 2).

Discussion

We predicted that tetraploids would show a more substantial reduction in growth rate under low-P conditions compared to their performance in high-P conditions than triploids if the high P content of nucleic acids imposes greater sensitivity to P limitation (Neiman et al. 2009, 2013). Consistent with these expectations, we found that growth rate in tetraploid *P. antipodarum* was much more negatively affected by dietary P scarcity than it was in triploids.

These results are the first to show experimentally that ploidy and dietary P can have an interactive effect on growth in animals. Benefits of polyploidy, including genomic buffering and greater potential for evolutionary innovation, have received considerable attention as factors explaining ploidy variation (e.g., Otto and Whitton 2000; te Beest et al. 2012). Although infrequently emphasized, nutrient and energy costs associated with nucleic acid production and maintenance may also influence polyploid evolution (Lewis 1985; Neiman et al. 2009, 2013); a similar argument has been offered for nutrient constraints on genome size evolution (Hessen et al. 2008, 2010). Evaluating these nutrient constraint hypotheses with comparisons between distantly related and/or allopatric taxa is difficult because potential differential effects of

P limitation are confounded by the many other differences likely to exist between organisms that are not close relatives and/or do not coexist. The recent identification of asexual tetraploids in *P. antipodarum* (Neiman et al. 2011, 2012; Liu et al. 2012) provides a rare opportunity to study ploidy effects in an experimentally tractable animal system while controlling for mode of reproduction. Our result showing an interaction between ploidy and sensitivity to P limitation suggests that variation in P availability warrants additional attention as a potential driver of ploidy variation.

We also found that triploid and tetraploid *P. antipodarum* differed in the sensitivity of their body P content to dietary P: while body P content of triploids was unaffected by P treatment, tetraploids experienced a significant drop in body P content under low-P conditions relative to high-P conditions. The parallel responses of specific growth rate and body P content in triploids and tetraploids under low-P conditions versus high-P conditions is consistent with the main prediction of the growth rate hypothesis (Elser et al. 2003): higher individual growth rates are driven by increased allocation of biomass to rRNA, which is rich in P relative to other major biomolecules in nonvertebrate animals. The higher growth rate and P content of tetraploids under high P is also consistent with the possibility that additional genome copies in tetraploids may allow them to produce more protein when P is adequate (e.g., DeMaggio and Lambrukos 1974; de Godoy et al. 2008).

Although we found a clear connection between ploidy and response of growth rate to P, much of the variation in growth rate in our study was explained by a main effect of dietary P. Similar major consequences of low dietary P content have been documented in other benthic snails (Stelzer and Lamberti 2002), including *P. antipodarum* (Tibbets et al. 2010). The major role of dietary P, along with the substantial within-ploidy level effects of lineage on growth rate, suggests that some of the impact of P scarcity on *P. antipodarum* is likely to be independent of ploidy differences. More broadly, although our data suggest that ploidy level can profoundly influence response to P limitation, more information is needed to determine the extent to which dietary P and ploidy can interact to influence competitive outcomes, especially under field conditions.

Alternative explanations for the patterns we observed cannot be excluded with the data at hand. One potential alternative mechanism for our findings involves behavioral interactions. In our experiment, we placed one snail from each of 25 lineages (20 triploid, 5 tetraploid) in each of 24 aquaria that we then assigned to either the low-P or high-P treatment. As a result, snails from each lineage interacted over the course of the experiment. In part, we chose this design over one where snails were isolated because the diverse groups of *P. antipodarum* used in our experiment provide a closer approximation to natural populations of *P. antipodarum*, which are very diverse (Fox et al. 1996; Jokela et al. 2003) and often high density (e.g., Schreiber et al. 1998). However, because this design does allow snails to interact, we cannot formally exclude the possibility that our results reflect an undocumented pattern of behavioral interaction (e.g., increased antagonistic interactions particularly detrimental to tetraploids under low-P conditions) and/or other potential phenotypic differences that may exist between triploids and tetraploids.

An important context for this work is the wide across-population variation in the relative frequency of sexual diploid and asexual polyploid *P. antipodarum* (Lively 1987; Lively and Jokela 2002) and triploid vs. tetraploid asexual *P. antipodarum* (Neiman et al. 2011). This variation is in large part responsible for the growing prominence of *P. antipodarum* as a model system for studying the maintenance and distribution of sex (e.g., Neiman et al. 2010; King et al. 2011). Because many mixed sexual/asexual animal systems feature diploid sexuals and polyploid asexuals (Suomalainen et al. 1987; Otto and Whitton 2000; Lundmark and Saura 2006), including *P. antipodarum* (Wallace 1992), resource costs related to the dietary phosphorus demands of nucleic acid production could potentially facilitate the persistence of sex when asexual taxa are polyploid and phosphorus availability limits important traits like growth and reproduction (Neiman et al. 2009, 2013).

The interactive effects of ploidy and dietary P on specific growth rate is one of several nonmutually exclusive mechanisms that could play a role in the generation and maintenance of across-population ploidy variation in *P. antipodarum*. For example, it is possible that the many potential genotypic and phenotypic consequences of polyploidization (e.g., Comai 2005; Otto 2007; Albertin and Marullo 2012) could vary in importance across populations. Patterns of across-population ploidy variation could also reflect relatively rare and/or recent origins of tetraploid *P. antipodarum* (Neiman et al. 2011).

Our study outcomes generate the testable prediction that across-population variation in the relative frequency of different ploidy levels will at least in part reflect variation in dietary P availability. *P. antipodarum* likely faces variable P limitation because it is found in diverse freshwater habitats (Winterbourn 1970a; Haase 2008); approximately 30% of New Zealand lakes are oligotrophic, and many New Zealand lakes have N : P ratios high enough that algal growth may be P-limited (Verberg et al. 2010). Along with the experimental tractability of the system, these features suggest *P. antipodarum* may be a useful system for examining the importance of resource-related disadvantages of polyploidy.

ACKNOWLEDGMENTS

We thank R. Bilka, T. Chamberlain, B. Hanson, J. Jokela, K. King, K. Klappert, K. Larkin, C. Lively, B. Rasmussen, R. Sanders, C. Schiltz,

J. Sharbrough, K. Theisen, A. Thompson, C. Tucci, A. Van Alst, P. Wilton, and N. Zachar for field collections and lab work. We thank J. Van Berkel and staff at the Kaikoura Field Station for logistical support. We also thank J. Dudycha and 2 anonymous reviewers for insightful comments on an earlier version of the manuscript. Funding for Neiman comes from the University of Iowa, Research Council of Norway (Project No. 196468/V40), and NSF-MCB 1122176. Funding for Kay is from NSF-DEB 0842038.

LITERATURE CITED

Albertin, W., and P. Marullo. 2012. Polyploidy in fungi: evolution after whole-genome duplication. Proc. R. Soc. Lond. B 279:2497–2509.

Arendt, J. D. 1997. Adaptive intrinsic growth rates: an integration across taxa. Q. Rev. Biol. 72:149–177.

Bennett, M. D., and I. J. Leitch. 2010. Angiosperm DNA *C*-values database (release 7.0, Dec. 2010). Available at www.kew.org/cvalues/. Accessed August 22, 2012.

Broekhuizen, N., S. Parkyn, and D. Miller. 2001. Fine sediment effects on feeding and growth in the invertebrate grazers *Potamopyrgus antipodarum* (Gastropoda, Hydrobiidae) and *Deleatidium* sp. (Ephemeroptera, Leptophlebiidae) Hydrobiologia 457:125–132.

Cavalier-Smith, T. 1978. Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. J. Cell Sci. 34:247–278.

Comai, L. 2005. The advantages and disadvantages of being polyploid. Nat. Rev. Genet. 6:836–846.

de Godoy, L. M. F., J. V. Olsen, J. Cox, M. L. Nielsen, N. C. Hubner, F. Fröhlich, T. C. Walther, and M. Mann. 2008. Comprehensive mass-spectrometry-based proteome quantification of haploid versus diploid yeast. Nature 455:1251–1254.

DeMaggio, A. E., and J. Lambrukos. 1974. Polyploidy and gene dosage effects on peroxidase activity in ferns. Biochem. Genet. 12:429–440.

Dorgelo, J., and P. E. G. Leonards. 2001. Relationship between C/N ratio of food types and growth rate in the snail *Potamopyrgus jenkinsi* (E. A. Smith). J. North Am. Benthol. Soc. 20:60–67.

Dorgelo, J., H. Meester, and C. van Velzen. 1995. Effects of diet and heavy metals on growth rate and fertility in the deposit-feeding snail *Potamopyrgus jenkinsi* (Smith) (Gastropoda: Hydrobiidae). Hydrobiologia 316:199–210.

Elser, J. J., K. Acharya, M. Kyle, J. Cotner, W. Makino, T. Markow, T. Watts, S. Hobbie, W. Fagan, J. Schade, et al. 2003. Growth rate-stoichiometry couplings in diverse biota. Ecol. Lett. 6:936–943.

Fankhauser, G. 1945. The effects of changes in chromosome number on amphibian development. Q. Rev. Biol. 20:20–78.

———. 1972. Memories of great embryologists: reminiscences of F. Baltzer, H. Spemann, F. R. Lillie, R. G. Harrison, and E. G. Conklin. Am. Sci. 60:46–55.

Fox, J. A., M. F. Dybdahl, J. Jokela, and C. M. Lively. 1996. Genetic structure of coexisting sexual and clonal subpopulations in a freshwater snail (*Potamopyrgus antipodarum*). Evolution 50:1541–1548.

Frost, P. C., J. P. Benstead, W. F. Cross, H. Hillebrand, J. H. Larson, M. A. Xenopoulos, and T. Yoshida. 2006. Threshold elemental ratios of carbon and phosphorus in aquatic consumers. Ecol. Lett. 9:774–779.

Gregory, T. R. 2005. Genome size evolution in animals. Pp. 3–87 *in* T. R. Gregory, ed. The evolution of the genome. Elsevier, Oxford, U. K.

———. 2012. Animal genome size database. Available at www.genomesize.com. Accessed August 22, 2012.

Haase, M. 2008. The radiation of hydrobiid gastropods in New Zealand: a revision including the description of new species based on morphology and mtDNA sequence information. Syst. Biodiv. 6:99–159.

Hair, J. B., and E. J. Beuzenberg. 1961. High polyploidy in a New Zealand *Poa*. Nature 189:160.

Hessen, D., M. Ventura, and J. J. Elser. 2008. Do phosphorus requirements for RNA limit genome size in crustacean zooplankton? Genome 51:685–691.

Hessen, D. O., P. D. Jeyasingh, M. Neiman, and L. J. Weider. 2010. Genome streamlining and the elemental costs of growth. Trends Ecol. Evol. 25:75–80.

Hicks, B. J. 1997. Food webs in forest and pasture streams in the Waikato region, New Zealand: a study based on analyses of stable isotopes of carbon and nitrogen and fish gut contents. NZ J. Mar. Freshwater Res. 31:651–664.

James, M. R., I. Hawes, M. Weatherhead, C. Stanger, and M. Gibbs. 2000. Carbon flow in the littoral food web of an oligotrophic lake. Hydrobiologia 441:93–106.

Jokela, J., C. M. Lively, M. F. Dybdahl, and J. A. Fox. 2003. Genetic variation in sexual and clonal lineages of a freshwater snail. Biol. J. Linnean Soc. 79:165–181.

King, K. C., J. Jokela, and C. M. Lively. 2011. Parasites, sex, and clonal diversity in natural snail populations. Evolution 65:1474–1481.

King, K. C., O. Seppälä, and M. Neiman. 2012. Is more better? Polyploidy and parasite resistance. Biol. Lett. 8:598–600.

Krist, A.C., J. Jokela, J. Wiehn, and C.M. Lively. 2004. Effects of host condition on susceptibility to infection, parasite development rate, and parasite transmission in a snail-trematode interaction. J. Evol. Biol. 17:33–40.

Leitch, I. J., and M. D. Bennett. 2004. Genome downsizing in polyploid plants. Biol. J. Linn. Soc. 82:651–663.

Leitch, A. R., and I. J. Leitch. 2008. Genomic plasticity and the diversity of polyploid plants. Science 320:481–483.

Levin, D. A. 1983. Polyploidy and novelty in flowering plants. Am. Nat. 122:1–25.

Lewis, W. M., Jr. 1985. Nutrient scarcity as an evolutionary cause of haploidy. Am. Nat. 125:692–701.

Liu, H.-P., R. Hershler, J. Marn, and T. M. Worsfold. 2012. Microsatellite evidence for tetraploidy in invasive populations of the New Zealand mudsnail *Potamopyrgus antipodarum* (Gray, 1843). J. Moll. Stud. 109: 57–62.

Lively, C. M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. Nature 328:519–521.

Lively, C. M., and J. Jokela. 2002. Temporal and spatial distributions of parasites and sex in a freshwater snail. Evol. Ecol. Res. 4:219–226.

Lundmark, M., and A. Saura. 2006. Asexuality alone does not explain the success of clonal forms in insects with geographical parthenogenesis. Hereditas 143:23–32.

Lynch, M. 2007. The origins of genome architecture. Sinauer Associates, Sunderland, U. S. A.

Mable, B. K., M. A. Alexandrou, and M. I. Taylor. 2011. Genome duplication in amphibians and fish: an extended synthesis. J. Zool. 284:151–182.

Neiman, M., A. D. Kay, and A. Krist. 2013. Can resource costs of polyploidy provide an advantage to sex? Heredity. doi: 10.1038/hdy.2012.78.

Neiman, M., K. Larkin, A. R. Thompson, and P. Wilton. 2012. Male offspring production by asexual *Potamopyrgus antipodarum*, a New Zealand snail. Heredity 109:57–62.

Neiman, M., K. Theisen, M. E. Mayry, and A. D. Kay. 2009. Can phosphorus limitation contribute to the maintenance of sex? A test of a key assumption. J. Evol. Biol. 22:1359–1363.

Neiman, M., G. Hehman, J. T. Miller, J. M. Logsdon, Jr., and D. R. Taylor. 2010. Accelerated mutation accumulation in asexual lineages of a freshwater snail. Mol. Biol. Evol. 27:954–963.

Neiman, M., D. Paczesniak, D. M. Soper, A. T. Baldwin, and G. Hehman. 2011. Wide variation in ploidy level and genome size in a New Zealand

freshwater snail with coexisting sexual and asexual lineages. Evolution 65:3202–3216.

Otto, S. P. 2007. The evolutionary consequences of polyploidy. Cell 131:452–462.

Otto, S. P., and A. C. Gerstein. 2009. Primer: the evolution of haploidy and diploidy. Curr. Biol. 18:R1121-R1124.

Otto, S. P., and J. Whitton. 2000. Polyploid incidence and evolution. Ann. Rev. Genet. 34:401–437.

Parisod, C., R. Holderegger, and C. Brochmann. 2010. Evolutionary consequences of autopolyploidy. New Phytol. 186:5–17.

Ramsey, J. 2011. Polyploidy and ecological adaptation in wild yarrow. Proc. Nat. Acad. Sci. USA 108:7096–7101.

Schreiber, E. S. G., A. Glaister, G. P. Quinn, and P. S. Lake. 1998. Life history and population dynamics of the exotic snail *Potamopyrgus antipodarum* (Prosobranchia: Hydrobiidae) in Lake Purrumbete, Victoria, Australia. Marine and Freshwater Research 49:73–78.

Stelzer, R. S., and G. A. Lamberti. 2002. Ecological stoichiometry in running waters: periphyton chemical composition and snail growth. Ecology 83:1039–1051.

Sterner, R. W., and J. J. Elser. 2002. Ecological stoichiometry: The biology of elements from molecules to the biosphere. Princeton Univ. Press, Princeton, NJ.

Suomalainen, E., A. Saura, and J. Lokki. 1987. Polyploidy in association with parthenogenesis. Pp. 71–112 in E. Suomalainen, A. Saura, and J. Lokki, eds. Cytology and evolution in parthenogenesis. CRC Press, Boca Raton, U. S. A.

te Beest, M., J. J. Le Roux, D. M. Richardson, A. K. Brysting, J. Suda, M. Kubešová, and P. Pyšek. 2012. The more the better? The role of polyploidy in facilitating plant invasions. Ann. Botany 109:19–45.

Tibbets, T. M., A. C. Krist, R. O. Hall, Jr., and L. A. Riley. 2010. Phosphorus-mediated changes in life history traits of the invasive New Zealand mudsnail (*Potamopyrgus antipodarum*). Oecologia 163:549–559.

van Geest, G. J., R. Sachse, M. Brehm, E. Van Donk, and D. O. Hessen. 2010. Maximizing growth rate at low temperatures: RNA:DNA allocation strategies and life history traits of Arctic and temperate *Daphnia*. Polar Biol. 33:1255–1262.

Verburg, P., K. Hamill, M. Unwin, and J. Abell. 2010. Lake water quality in New Zealand 2010: Status and Trends. New Zealand Ministry for the Environment: National Institute of Water & Atmospheric Research Ltd., Client Report.

Wallace, C. 1992. Parthenogenesis, sex, and chromosomes in *Potamopyrgus*. J. Moll. Stud. 58:93–107.

Weeks, A. R., F. Marec, and J. A. J. Breeuwer. 2001. A mite species that consists entirely of haploid females. Science 292:2479–2482.

Winterbourn, M. J. 1970a. The New Zealand species of *Potamopyrgus* (Gastropoda: Hydrobiidae). Malacologia 10:283–321.

Winterbourn, M. J. 1970b. Population studies on the New Zealand freshwater gastropod *Potamopyrgus antipodarum* (Gray). Proc. Malacolog. Soc. Lond. 39:139–149.

Associate Editor: J. Dudycha

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Mean specific growth rate (initial length residuals; 95% CI) in the high and low-P treatments for the 20 triploid line-ages (left) and the five tetraploid lineages (right).

This material is available as part of the online article form:
http://www.blackwell-synergy.com/doi/abs/XXXXX (This link will take you to the article abstract).