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SHORT COMMUNICATION

Can phosphorus limitation contribute to the maintenance of sex? A test of a key assumption

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Abstract

Why sex is so common remains unclear; what is certain is that the predominance of sex despite its profound costs means that it must confer major advantages. Here, we use elemental and nucleic acid assays to evaluate a key element of a novel, integrative hypothesis considering whether sex might be favoured because of differences in body composition between sexuals and asexuals. We found that asexual *Potamopyrgus antipodarum*, a New Zealand snail, have markedly higher bodily phosphorus and nucleic acid content per unit mass than sexual counterparts. These differences coincide with and are almost certainly linked to the higher ploidy of the asexuals. Our results are the first documented body composition differences between sexual and asexual organisms, and the first detected phenotypic difference between sexual and asexual *P. antipodarum*, an important natural model system for the study of the maintenance of sex. These findings also verify a central component of our hypothesis that competition between diploid sexuals and polyploid asexuals could be influenced by phosphorus availability.

Introduction

Why sexual reproduction is so common in spite of its profound costs is considered to be one of the most important unanswered questions in evolutionary biology (Bell, 1982). The 'cost of males' is one faced, at least in principle, by all dioecious sexuals (Maynard Smith, 1978). This cost is defined in terms of the difference in the rate of daughter production in sexual vs. asexual females: all else being equal, asexual females will produce twice as many daughters as sexual females, which make both male and female offspring. Because only females contribute directly to the rate of population growth, the production of males creates a two-fold cost of sexual reproduction that should logically result in the selective elimination of sex (Williams, 1975; Maynard Smith, 1978).

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The predominance of sex means that there must be differences between sexuals and asexuals that confer immediate advantages to sexual individuals. Most work on potential benefits of sex has focused on genetic consequences of recombination, such as disruption of linkage disequilibria, clearing deleterious mutations, and producing genetically variable offspring (reviewed in Kondrashov 1993; Barton & Charlesworth, 1998; de Visser & Elena, 2007). However, the cost of sex can be mitigated even in the absence of benefits of recombination if there are phenotypic disadvantages associated with asexuality (Maynard Smith, 1978).

Disadvantages tied to asexuality may arise when asexuals have higher ploidy levels than sexual counterparts, which is very often the case (Suomalainen *et al.*, 1987; Otto & Whitton, 2000). While higher ploidy could confer both phenotypic and genetic benefits (Otto & Whitton, 2000), polyploidy may also be costly. We hypothesize that higher ploidy could also confer resource-based costs associated with increased investment in nucleic acids that could in turn affect competitive outcomes between sexuals and asexuals. Some precedent for this idea comes from Lewis (1985), who argued that the metabolic cost and high carbon and phosphorus (P) content of DNA meant that reduced ploidy levels might be favoured in single-celled organisms if it reduced nutrient requirements in nutrient-poor conditions.

Allocation to nucleic acids may increase requirements for dietary P because (i) P content in nucleic acids (~9%) is much higher than that in other major biomolecules (Sterner & Elser, 2002), (ii) nucleic acids (primarily rRNA) often comprise a large, but extremely variable, fraction of organismal dry mass (Elser *et al.*, 1996; Sterner & Elser, 2002; Weider *et al.*, 2005; Elser, 2006), and (iii) dietary P requirements are closely tied to bodily P content (Sterner & Elser, 2002; Frost *et al.*, 2006). The association between nucleic acid content and P requirements is likely to be particularly close in invertebrates because P in rRNA generally accounts for a large fraction of total body P content in these organisms (Elser *et al.*, 2003). Indeed, models and empirical work that combine information on energetics and body composition suggest that body P content is a key determinant of sensitivity to P limitation in invertebrates (Sterner & Elser, 2002; Frost *et al.*, 2006).

An association between ploidy level and P requirements leads to our predictions that asexual polyploids might be more limited by environmental P availability than sexual counterparts, and that the outcome of competition between asexual and sexual taxa that differ in ploidy might be directly influenced by the availability of P. The implications are that the 'paradox' of sex could be ameliorated by the specific resource requirements associated with higher ploidy. There is a large body of research demonstrating that key ecological and evolutionary processes are influenced by the interplay between environmental P availability and body composition (Sterner & Elser, 2002), but no previous study has considered the maintenance of sex from this perspective.

A key assumption of our hypothesis that asexual polyploids will be more sensitive to limited P than sexual diploids is that asexual polyploids contain higher bodily nucleic acid and P content than sexuals with lower ploidy levels. Higher ploidy is generally associated with higher per-cell DNA content, but very little is known about whether higher DNA and especially RNA content are higher *per unit biomass* in polyploids. The focus on RNA is important because RNA generally makes up a much larger fraction of invertebrate biomass than does DNA, while focus on mass fraction is critical because this measure is a key indicator of how organisms allocate scarce resources (Sterner & Elser, 2002). Data relating ploidy level and nucleic acid content are particularly scarce for animal taxa.

We tested this assumption by comparing bodily phosphorus and nucleic acid content in sexual diploid vs. asexual triploid *Potamopyrgus antipodarum* (Wallace, 1992), a freshwater snail native to New Zealand that has become a model system for studying the maintenance of sex. Because sexual and asexual *P. antipodarum* coexist within some lake populations (Lively & Jokela, 2002), this system provides an ideal opportunity for assessing the phenotypic consequences of polyploidy in the context of sex. We found that asexual *P. antipodarum* have markedly higher bodily P and nucleic acid content per unit dry mass than their sexual counterparts. This constitutes, to the best of our knowledge, the first documented example of body composition differences between sexual and asexual organisms, and the first clear phenotypic difference between sexual and asexual *P. antipodarum*.

Materials and methods

Body composition assays

Bodily P content was measured in 230 adult female *P. antipodarum* and bodily RNA and DNA content was measured in an additional 154 adult females. Snails were sampled from 13 laboratory populations (three sexual and nine asexual) and five New Zealand lake populations; at least 12 snails were assayed from each population (at least six for each of the two assays). Laboratory populations were descended from snails originally collected from various New Zealand lakes. Two asexual populations were descended from invasive European populations. All laboratory snails were fed identical diets (*ad lib Spirulina* algae) and reared at 17 °C on a 16 h day/8 h night regime.

Lake snails were sampled in January, 2008 from five New Zealand lakes: Hawdon, Ianthe, Lady, Mapourika and Alexandrina. The snails were kept alive until March 2008, when flow cytometry was used on the heads of 40 female snails per lake to determine their sexuality (Osnas & Lively, 2006). All Ianthe and Lady snails were sexual, while >75% snails from the other three lakes were asexual. Only asexual snails were used for P and nucleic acid assays for these three populations.

Chemical assays were performed on snails removed from shells (headless bodies for lake snails): preliminary analyses indicated shells contained no measurable P. Bodily P content was measured using potassium persulfate and sulfuric acid digestion followed by ascorbate-molybdate colorimetry and with a Flow Solution IV autoanalyzer (OI Analytical, College Station, TX, USA). The RNA and DNA quantification assay followed Kyle *et al.* (2003). The DNA and RNA estimates per wet mass were converted to estimates per dry mass using a previously established relationship between wet and dry mass in sexual vs. asexual *P. antipodarum* (data not shown).

Statistical analyses

Whole-snail body percentage of P, RNA and DNA log–log dry mass residuals were calculated separately for lake vs. laboratory snails to correct for the use of headless bodies for P and nucleic acid assays for lake snails. Residuals were compared using 'proc glm' in SAS 9.1.3 (SAS

Institute, Cary, NC, USA); population was a random factor nested within the fixed factor of sexuality, and population type (laboratory vs. lake) and sexuality were included as main effects. Linear regression was used within SPSS (SPSS Statistics 17.0, SPSS Inc., Chicago, IL, USA) to compare mean population whole-snail nucleic acid (RNA + DNA) content to mean population whole-snail P content.

Results

Asexual *P. antipodarum* had significantly higher bodily P content ($F_{1,15} = 8.44$, $P = 0.009$), RNA content ($F_{1,15} = 17.43$, $P < 0.001$) and DNA content ($F_{1,15} = 16.53$, $P = 0.001$) than sexual diploid snails (Fig. 1). Snail source (laboratory vs. lake) did not affect P content ($F_{1,173} = 0.69$, $P = 0.406$), RNA content ($F_{1,128} = 0.46$, $P = 0.497$), or DNA content ($F_{1,128} = 0.37$, $P = 0.547$). There were also significant differences among asexual populations in bodily P content ($F_{12,129} = 3.62$, $P < 0.001$), RNA content ($F_{12,93} = 4.451$, $P < 0.001$), and DNA content ($F = 4.995$, $P < 0.001$). Sexual populations also differed in P content ($F_{4,82} = 3.37$, $P = 0.008$) and DNA content ($F_{4,82} = 7.63$, $P < 0.001$), but not RNA content ($F_{4,82} = 2.07$, $P = 0.105$). Across all snails, there was a significant positive association between mean population P content and nucleic acid content (Fig. 1; $r^2 = 0.59$, $P < 0.001$). Results were similar when the analysis was restricted to asexual populations ($r^2 = 0.52$, $P = 0.005$). Assuming G-C content is 40%, as in some aquatic invertebrates (Ventura, 2006), P mass in DNA and RNA was estimated to be 0.89 and 0.85 m m^{-1}, respectively. This means that across all populations, a substantial fraction of body P was contained in nucleic acids (mean across all populations ± SE: RNA = 42.61% ± 2.34; DNA = 21.37% ± 1.08).

Discussion

We found that asexual triploid *P. antipodarum* had significantly higher bodily phosphorus, RNA, and DNA content per unit mass than their sexual diploid counterparts, and that these differences existed in both laboratory-raised and field-collected samples. We also found a strong positive association between nucleic acid content and P content across all lineages, and that a majority of body P content was accounted for by nucleic acids. Taken together, these data suggest that greater allocation to nucleic acids in asexual triploids leads to higher bodily P content. To the best of our knowledge, this represents the first documented example of differences in body composition between sexual vs. asexual organisms and the first consistent phenotypic divergence in sexual vs. asexual *P. antipodarum* (Jokela *et al.*, 1997, 2003).

The higher P content in asexual *P. antipodarum* means that ploidy affects body composition in a manner that could ameliorate the cost of sex. There is a large body of recent literature suggesting that body P content is likely

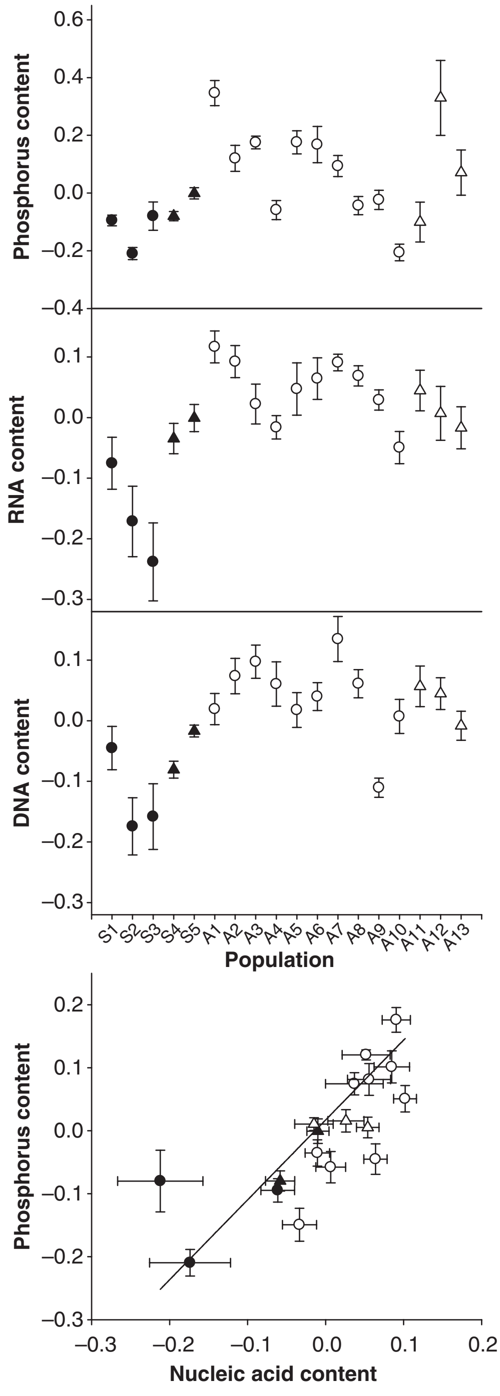

Fig. 1 Body composition in sexual (black) vs. asexual (white) *Potamopyrgus antipodarum*. All values are log-transformed residuals of log-transformed dry weight. Circles and triangles indicate lab and lake populations, respectively. Error bars represent SE of population mean.

to play an important role in ecological and evolutionary processes operating within natural populations (Sterner & Elser, 2002; Elser *et al.*, 2006). Our data verify a key element of our hypothesis that greater sensitivity to P availability in asexual polyploids may diminish competitive or demographic advantages over sexual diploids when P is scarce. The implications are that the persistence of sexual taxa in many systems and some of the well-characterized distributional differences between sexual vs. asexual animal taxa (Suomalainen *et al.*, 1987) as well as in polyploids vs. diploids (Bierzychudek, 1985) could be linked to ploidy level and its effect on body composition.

General applicability of this hypothesis to the maintenance of sex requires demonstration that (i) asexual polyploids in multiple taxa have higher nucleic acid and P content than sexual counterparts with lower ploidy and (ii) high P content in these asexuals is consistently associated with greater sensitivity to P limitation. For *P. antipodarum*, experiments examining the effect of P limitation on fitness and the outcome of competition between sexual and asexual snails, as well as studies of dietary P availability in natural populations, are the next steps in assessing whether the body composition differences could be of relevance to the maintenance of sex in this system. Our hypothesis does not attempt to explain the association between ploidy and asexuality seen in many taxa. In addition, it must be emphasized that our hypothesis posits that P limitation acts as one influence on competitive outcome between asexual and sexual taxa that differ in ploidy, and would be likely to work in concert with other benefits associated with sex (i.e. West *et al.*, 1999). Nevertheless, the hypothesis provides a conceptual advance by proposing that traits associated with asexuality in many taxa but that are not direct consequences of sexual reproduction could be relevant to the maintenance of sex. Our results indicate that a key element of this hypothesis is verified in a natural model system.

We also found evidence for widespread variation in body composition within and among sexual and asexual *P. antipodarum* originating from many different populations. To the best of our knowledge, our study represents the most extensive intra-specific survey of variation in bodily P and nucleic acid content ever conducted. Recent studies find evidence for wide intra-specific variation in P content (DeMott *et al.* 2004), and suggest that the rate of allele frequency change (Gorokhova *et al.*, 2002) and the mutation rate (Shufran *et al.*, 2003) at the nuclear ribosomal RNA loci that are a main source of variation in bodily RNA and P content can be extremely rapid. This might contribute to the wide variation in body composition amongst asexual *P. antipodarum* lineages we surveyed, which are of polyphyletic origin (Dybdahl & Lively, 1995; Neiman & Lively, 2004) and often quite genetically distinct from one another (Neiman *et al.*, 2005). Another potential explanation is changes in genome structure or size, which might be associated with selection for phosphate economy (Elser *et al.*, 2000; Cavalier-Smith, 2005; Hessen *et al.*, 2008).

Outside of the context of sex, our data point to a positive association between ploidy and bodily RNA content. This result demonstrates that whole-genome silencing does not occur in the triploids, a topic of broad interest that has received a great deal of recent attention (e.g. Pala *et al.*, 2008). Beacuse *P. antipodarum* is an autopolyploid (Dybdahl & Lively, 1995), this finding is in line with recent data from plants suggesting that reports of dosage compensation in allopolyploids may be due more to hybridization than polyploidy *per se* (Adams, 2007).

In conclusion, we used an extensive intra-specific study of body composition to characterize a novel phenotypic difference between asexual vs. sexual organisms that could be relevant to the maintenance and distribution of sex. These results are also relevant to important basic questions in ecological stoichiometry, such as the extent to which body composition varies within species, and provide new insights into the relationship between ploidy level and gene expression.

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