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Functional and ecological significance of rDNA intergenic spacer variation in a clonal organism under divergent selection for production rate

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It has recently been hypothesized that variation in the intergenic spacer (IGS) of rDNA has considerable developmental, evolutionary and ecological significance through effects on growth rate and body C : N : P stoichiometry resulting from the role of the IGS in production of rRNA. To test these ideas, we assessed changes in size and structure of the repetitive region of the IGS, juvenile growth rate (JGR), RNA and phosphorus (P) contents in clonal lineages of *Daphnia pulex* derived from a single female and subjected to divergent selection on weight–specific fecundity (WSF). As a result of selection, WSF diverged rapidly, with significant reductions within two generations. Other significant changes accompanying shifts in WSF were that juveniles produced by low-WSF females grew more rapidly and had higher RNA and P contents. An increased predominance of long IGS variants was observed in lineages with elevated JGRs and low WSF. The observed variations in IGS length were related to the number of subrepeat units carrying a promoter sequence in the repetitive region. These results strongly support the hypothesized relationships, indicate a genetic mechanism for the evolution of such associations and demonstrate that *Daphnia* (and perhaps other parthenogens) possess considerable potential for rapid adaptive change in major life-history traits.

Keywords: *Daphnia*; asexual lineages; rDNA intergenic spacer structure; RNA; phosphorus; growth rate

1. INTRODUCTION

In most eukaryotes, rDNA is arranged in tandemly repeated units containing the genes for the 18S, 5.8S and 28S rRNAs separated by spacers. The large intergenic spacer (IGS; formerly NTS—non-transcribed spacer) separating the 28S and 18S genes is itself internally repetitious, each repeat containing a tandem array of short subrepeat units (Reeder 1990; Paule & Lofquist 1996). The IGS is the most rapidly evolving region of rDNA, with the number and organization of internal repeats being species-specific and often varying between populations, individuals and even within a single cell (Paule & Lofquist 1996). Alterations in IGS repeats are thought to occur mostly by unequal crossing over during both sexual and asexual reproduction or in somatic cell lineages (Cullis & Charlton 1981; Gantley & Scott 1998). As a result, these variations in the structure of repetitive regions are common in many taxa and have been widely used in phylogenetic analysis and for quantification of gene flow between populations (Gray & Schnare 1996; Paule & Lofquist 1996).

Regulatory elements in the IGS, such as promoters, enhancers and terminators, play an essential part in the control of rRNA transcription (Reeder 1984; Flavell 1993), in processing of the transcript and probably in replication of the unit (Reeder 1990; Alvares *et al.* 1998; Marrocco *et al.* 1998). Variation in the length and structure of the IGS often results from variation in the number of subrepeat units and, consequently, in copy number of the regulatory elements (Reeder 1990; Moss & Stefanovsky 1995). Therefore, the molecular basis for IGS length variability is of particular interest because such variation may affect transcription efficiency and gene regulation *in vivo*. However, although there is solid evidence for the functional significance of IGS elements and for the existence of substantial variation in IGS size and structure within species and populations, fundamental questions regarding the effects of this variation on growth (Delany & Krupkin 1999) and development (Cluster *et al.* 1987) remain unanswered, and effects on life-history variables and ecological dynamics are as yet unexplored. It has recently been hypothesized that variation in the IGS has considerable developmental, evolutionary and ecological significance through effects on growth rate and body C : N : P stoichiometry resulting from the role of the IGS in rDNA expression and, therefore, production of phosphorus (P)-rich rRNA (Elser *et al.* 2000*a*). However, if direct or indirect natural selection on growth rate acts on genetic change associated with ribosomal genes because of the high RNA demands of rapid growth, then there would also be alterations in organismal C : N : P stoichiometry (Elser *et al.* 1996) due to increased allocation to P-rich rRNA (the 'growth rate hypothesis'). Furthermore, because differences in body C : N : P of consumers can affect the food-quality demands of herbivores (Elser *et al.*

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2000*b*) as well as rates and ratios of consumer-driven nutrient recycling in ecosystems (Elser & Urabe 1999), evolutionary processes that result in animals with elevated RNA contents and low C : P will have major impacts on ecosystem processes (Elser *et al.* 2000*a*).

To test these connections, we devised a strategy based on four related ideas and observations. First, parthenogenic and self-pollinating species can maintain substantial genotypic and phenotypic variability (Hebert & Crease 1980; Vrijenhoek 1998), including intraclonal variability (Lushai & Loxdale 2002; however see Lynch 1985), and clonal lineages have been found to be responsive to selection on morphological, physiological and life-history traits (Wilhoit & Mittler 1991; Andrade & Roitberg 1995). It has also recently been shown that apomictic parthenogenic lineages may have high intraclonal rDNA variation (Fenton *et al.* 1998; Mandrioli *et al.* 1999). Second, organisms exhibit increased RNA levels (mostly due to rRNA) when growing actively, as rapid protein synthesis by ribosomes is required to support fast growth (Alberts *et al.* 1983). Third, in organisms lacking major storage of P, higher specific growth rates are associated with increased body P content due to increased levels of P-rich rRNA (Elser *et al.* 1996, 2000*a*). Finally, under a selection regime that increases growth rate, IGS length would increase, as the rate of transcription of pre-rRNA would be directly proportional to the number of enhancers in the IGS (Cluster *et al.* 1987; Grimaldi & Di Nocera 1988). We were interested in the possibility of intraclonal variation in *Daphnia*, an ecologically significant herbivore in aquatic ecosystems. rDNA variation in *Daphnia* is extensive, with the number of IGS repeat types varying even among individuals that are identical with respect to allozyme and mitochondrial DNA markers (Crease & Lynch 1991; Crease 1995), indicating possible intraclonal heterogeneity. Moreover, this organism exhibits considerable phenotypic diversity despite extensive reliance on apomictic reproduction, making it a suitable model system for examining the relationship between selection, rDNA genotype and growth rate in the absence of meiotic recombination.

2. MATERIAL AND METHODS

(a) Daphnia *cultures and test samples*

Stock specimens of an obligately apomictic clone of *Daphnia pulex* were obtained from wild populations from Lake 110 in the Experimental Lakes Area (Ontario, Canada) and maintained in the laboratory for *ca*. 1 year. The identity of the species has been checked by allozyme electrophoresis. In all experiments, females were held individually in polyethylene jars containing 100 ml of zooplankton COMBO medium (Kilham *et al.* 1998) and fed chemostat-grown *Scenedesmus obliquus* (2.2–2.7 mg C l^{-1}; C : N : P *ca*. 120 : 18 : 1, by atoms) *ad libitum*. Culture medium and food were replaced every other day and all jars were kept at 21 ± 2 °C with an ambient light : dark cycle.

In all assays, individual dry weight (DW) in mg was determined from body length (BL) measurements in mm (from the base of the tail spine to the top of the head) according to length–weight regressions developed for this particular clone: $DW = 0.007BL^{2.23}$ ($r^2 = 0.92$) for juveniles and $DW = 0.0084BL^{2.58}$ ($r^2 = 0.90$) for adults. Egg measurements were taken within a few hours of the deposition of a new clutch of eggs into the brood pouch (i.e. egg stage I according to the criteria of Threlkeld (1979)), and their largest dimension was measured to the nearest 0.01 mm under a dissecting microscope. Biomass of progeny was calculated as the total biomass of neonates of the clutch. Weight-specific fecundity (WSF) was calculated as the ratio of the cumulative biomass of progeny produced during the first 14 days of a female's life to the female's biomass at the end of this period.

(b) *Artificial selection experiment*

Continuous disruptive selection on WSF was imposed on the progeny of a single female of *D. pulex*. Two selection lines (H and L) and a control line (C) were established by raising the offspring of the third clutch of a stem mother and allowing them to reproduce. All neonates were removed within 24 h of their release, counted and discarded. Selection proceeded by choosing three replicate parents with the highest (line H) and lowest (line L) WSF values. The control line, with the same number of replicates, was established by random selection. These selected females became progenitors of each selected line, and their next clutches were allowed to grow and reproduce. After scoring WSF in each subsequent selection round, three appropriate individuals (i.e. depending on the selection regime for that line: H, L or C) from the total pool of offspring in each line were chosen to continue the line. The selection procedure was repeated for four generations. After each selection round, all females were transferred to 1.5 ml microcentrifuge tubes, and stored at −80 °C until genetic analysis.

(c) *Early life-history traits*

In order to establish the link between WSF and growth performance during the juvenile period, it was necessary to assess early life-history traits in life-table experiments. The fifth generation of each line produced in the selection experiment was used to determine neonate body length (NBL) (mm), juvenile growth rates (JGR) (d^{-1}), size at first reproduction (SFR) (mm), age at first reproduction (AFR) (days) and size of the first clutch (eggs per female). In the present study, maturity of the female was operationally defined as the point when it deposited the first clutch of eggs into the brood pouch; hence, SFR and AFR were defined as the daphnid's size and age at maturity. Consequently, JGR was calculated as:

$$\mathrm{JGR} = \frac{\ln W_\mathrm{p} - \ln W_\mathrm{n}}{\mathrm{AFR}},$$

where W_p and W_n are the primapara and neonate weights, respectively.

Neonates of the third clutch were separated from females within 3 h of their release and grown until the primapara stage. Five to seven neonates from the same brood of the fourth-generation female (three lines with three females each) were chosen randomly to estimate their initial size; these individuals were also used for RNA measurements. Daphnids were checked once a day for the first 4 days and every 4–6 h during the rest of the experiment, and their reproductive status was recorded. When the first clutch was observed in the brood chamber, it was assumed to have been extruded from the ovaries in the middle of the period following the previous check, and AFR was calculated accordingly.

(d) *RNA content*

Microplate fluorometric assay with the RiboGreen RNA Quantitation Kit (Molecular Probes, Inc.) was performed to

quantify RNA in neonates after extraction with N-lauroysarcosine (Sigma, Inc.) followed by RNase digestion (RNase, DNase-free; 50 Kunitz U ml^{-1} in TE buffer; Boehringer Mannheim, Inc.), as described elsewhere (Gorokhova & Kyle 2002). The length of neonates from each selected line (fifth generation, third clutch, five to seven replicate neonates as repeated measurements for a female, three females per line) was measured, and then they were preserved individually in 1.5 ml microcentrifuge tubes with 10 µl RNA*Later* (Ambion, Inc.), and stored at 4 °C until analysis.

(e) *Phosphorus content*

Neonates (fifth generation, clutch five to seven, 20–25 individuals per sample) were placed onto pre-combusted and pre-weighed GF/C filters with three replicates for each female. Samples were dried at 60 °C for 24 h, weighed, and total P was determined using persulfate oxidation followed by analysis of orthophosphate using the acid molybdate technique (APHA 1992).

(f) *Analysis of intergenic spacer rDNA variation*

Total cellular DNA was extracted from females collected in the selection experiment (single individuals, 50–70 µg DW) using the Chelex method (Straughan & Lehman 2000). PCR reactions were carried out using a pair of primers designed to amplify the IGS region containing 330 bp subrepeats. The forward primer (5′ TGACGGGCGAAGCGAGACAG 3′) was positioned upstream of the first subrepeat and the reverse primer (5′ GACGGTGTGACGGCTTCTCT 3′) was positioned downstream of the last subrepeat (Crease 1993). Each PCR reaction contained 10–30 ng of template DNA and 10 pmol of each primer in a total volume of 50 µl. Amplification conditions were: 20 cycles at 94 °C for 30 s, 60 °C for 30 s, 70 °C for 6 min; then 10 cycles of 94 °C for 30 s, 60 °C for 30 s, 70 °C for 6 min with 20 s auto-extension; and a final extension at 70 °C for 7 min. PCR amplification products were separated in 1.2% (w/v) agarose gels at 70 V in TBE buffer. Genotype (i.e. presence or absence of specific bands) was observed after staining the gel with 1 µg ml^{-1} ethidium bromide. Samples were randomized within and between gels. Representative amplification products were cloned with the TOPO TA cloning kit (Invitrogen), and plasmid inserts were amplified with T3 and T7 primers. Complete sequences were obtained for each unique PCR fragment using an ABI 377 automated sequencer, aligned with the *D. pulex* IGS sequence from GenBank (accession number L07948) and submitted to GenBank (accession numbers AF456327 and AF456328).

(g) *Statistics*

Statistical tests were performed with GraphPad Prism, v. 3.0 (GraphPad Software). Significant outliers were detected using Grubb's test and removed from the calculations. Comparisons of three or more groups were performed with a one-way ANOVA with Bartlett's test for equal variances followed by Tukey's multiple comparison test.

3. RESULTS

(a) *Weight-specific fecundity*

Selection for low WSF produced a direct response and resulted in rapid divergence of the L line from the H and C lines (figure 1*a*). By the second generation, WSF of the L line was significantly lower than that of the H line

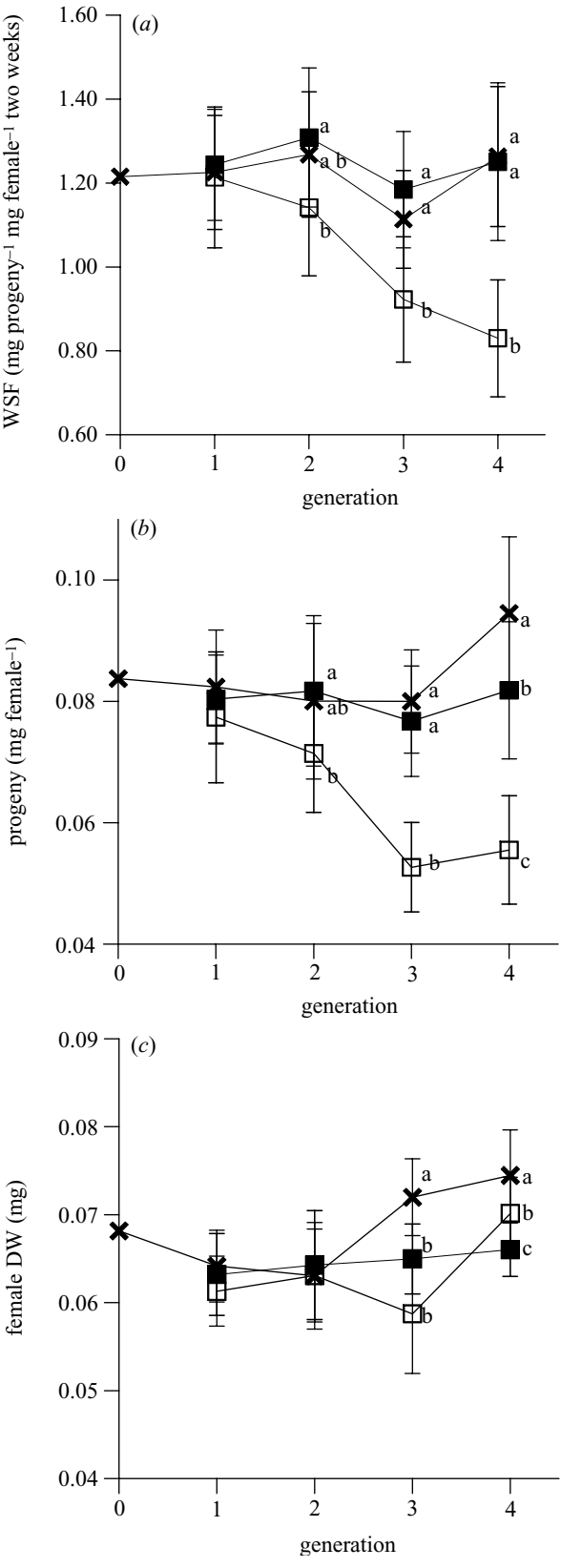

Figure 1. Response to selection for WSF in four generations of parthenogenically reproducing *D. pulex*: (*a*) WSF; (*b*) progeny biomass; and (*c*) female size. Open symbols, lines selected for low WSF; closed symbols, lines selected for high WSF; crosses, control lines. For each parameter, mean ± s.e. values are shown. Total number of individuals per line varied between 29 and 48. Unmatched letters indicate significant differences between the selection regimes within a group.

Table 1. Early life-history traits (mean ± s.d.) for individuals from the fifth generation of selected and control lines. (ED, egg diameter. One-way ANOVA and post-hoc Tukey's test ($^{*}p < 0.05$, $^{**}p < 0.01$). Values within a column that are not marked by asterisks are not significantly different.)

selection regime	ED (mm)	NBL (mm)	AFR (days)	SFR (mm)	first clutch (eggs per female)
low WSF	0.18 ± 0.01	0.61 ± 0.024	6.1 ± 0.21**	1.62 ± 0.11	2.3 ± 1.25*
high WSF	0.18 ± 0.02	0.60 ± 0.025	6.8 ± 0.32	1.56 ± 0.09	6.0 ± 2.10
control	0.19 ± 0.01	0.59 ± 0.034	6.9 ± 0.22	1.61 ± 0.10	5.0 ± 1.80

($F_{2,101} = 4.91$, $p < 0.01$), and by the third generation it significantly differed from the control (figure 1*a*; $F_{2,107} = 18.5$, $p < 0.001$). By contrast, no significant direct response was detected when selecting for high WSF (H line; figure 1*a*). Thus, the observed response was asymmetrical; the difference between selected lines resulted mainly from a significant decrease in offspring production in the L line (figure 1*b*; fourth generation: $F_{2,98} = 50.3$, $p < 0.001$). While there were some changes in female size during the experiment (figure 1*c*), the trend was not consistent and, therefore, did not affect the main result. In addition, the observed decrease in WSF was not due to differences in either egg (table 1; $F_{2,44} = 1.88$, $p > 0.2$) or neonate size (table 1; $F_{2,42} = 1.94$, $p > 0.2$) between the lines.

(b) *Early life-history traits*

Significant alterations in early life-history traits accompanied the changes in WSF during the selection experiment, i.e. indirect effects of selection were observed. As assayed by life-table studies using fifth generation animals (table 1), selection for low WSF significantly reduced AFR ($F_{2,6} = 10.77$, $p < 0.01$) and size of the first clutch ($F_{2,6} = 6.19$, $p < 0.03$), while no significant effect on SFR was observed ($F_{2,6} = 0.38$, $p > 0.7$). Because size at maturation was unaffected but juveniles reached that size more quickly in the low WSF lineages, JGR was significantly higher in the L line than in the H and C lines (figure 2; JGR: $F_{2,6} = 6.86$, $p < 0.03$). The number of juvenile instars did not vary: all females matured in five instars. Thus, by selecting for low WSF, we created a line that had accelerated somatic growth during the juvenile period and low egg production during the first adult instars.

(c) *RNA and phosphorus content*

In the fifth generation, neonates with high JGR from the L lineages had significantly higher RNA content than did the C and H lines (figure 2; RNA: $F_{2,6} = 6.13$, $p < 0.03$) and significantly higher P content than the H line (figure 2; P: $F_{2,6} = 6.03$, $p < 0.03$). Thus, the indirect effect of our selection regime on JGR was associated with significant changes in juvenile RNA and P contents.

(d) *Structural variations in intergenic spacer*

The genetic basis of these changes was examined by characterizing the length and structural variations in the rDNA IGS of the experimental animals using PCR-based methods. Two patterns of length variants were observed (figure 3*a*,*b*): (i) a 'single-band' type, with a *ca*. 1.45 kb fragment corresponding to the sequence containing four 330 bp subrepeats previously documented in the *D. pulex*

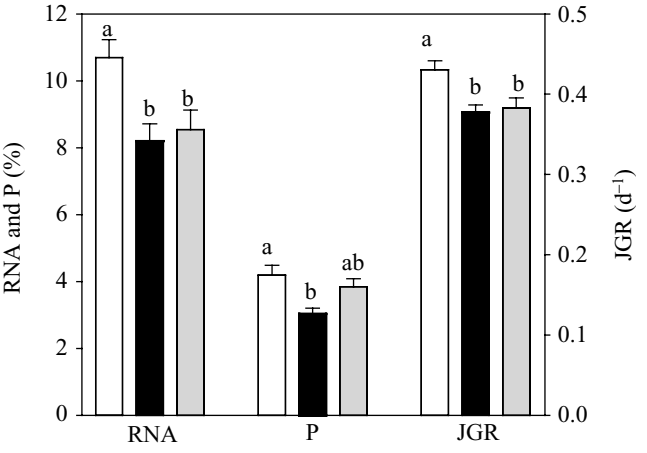

Figure 2. Indirect response to selection for WSF in fifth generation individuals: alterations in JGR, RNA and P concentrations in the selected (high WSF (black bars) and low WSF (white bars)) and control (grey bars) lines (mean ± s.e., three replicate lineages per line). Overall mean values and their corresponding standard errors were obtained for each line using average values derived from five to seven measurements for each replicate and, thus, dismissing intraclutch variation. For each parameter, unmatched letters indicate significant differences between the selection regimes.

IGS (Crease 1993); and (ii) a 'double-band' type with an additional *ca*. 0.92 kb fragment corresponding to the sequence containing only the first two 330 bp subrepeats. Each repeat contains a sequence (5′ TATATAGGGAAG; figure 3*a*) that was suggested to be a putative RNA polymerase promoter, such as is commonly found in IGS of other organisms (Crease 1993). The single-band type predominated, and in no case did individuals lack the 1.45 kb fragment (figure 3*b*). The double-band type indicates that there is length heterogeneity in the IGS among the rDNA arrays carried by a single individual. The 1.45 and 0.92 kb fragments occurred in the foundress and in *ca*. 40% of her offspring in the first generation. A striking feature of the low WSF–high JGR phenotype in the fourth generation was the low frequency of the 0.92 kb fragments compared with their frequency in the H and C lines. Indeed, *ca*. 80% of L line animals had only the 'single-band' type of IGS, while 41% of the C and 63% of the H line animals had the double-banded type of IGS (figure 3*c*).

4. DISCUSSION

In clonal lineages of *D. pulex* derived from a single female and subjected to divergent selection on WSF, IGS length variation was associated with significant changes in

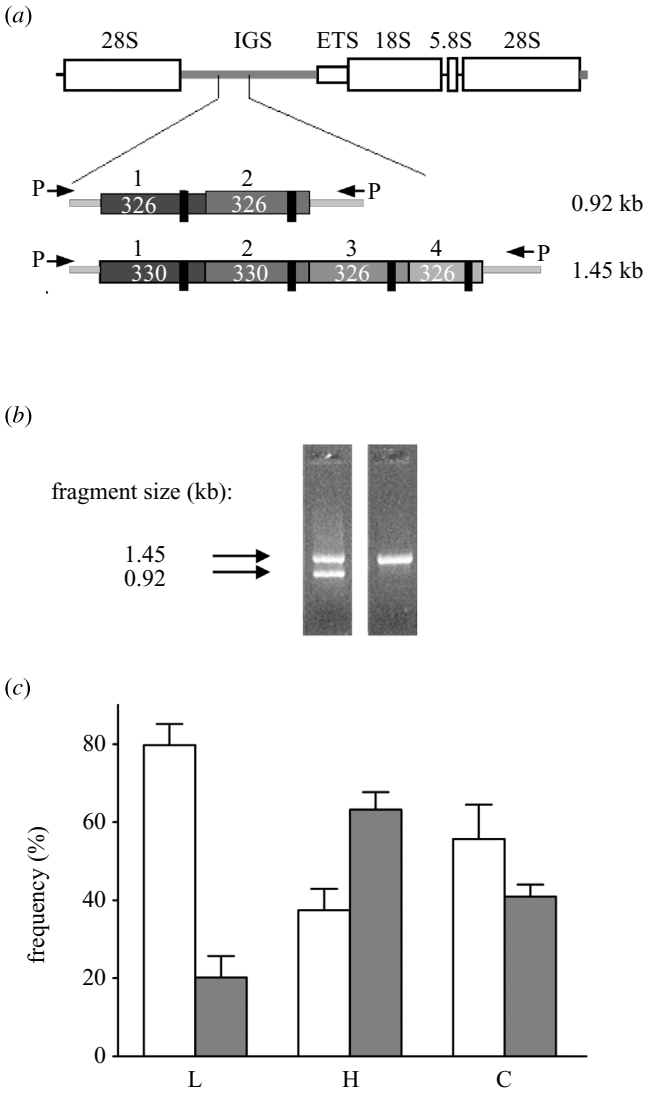

Figure 3. IGS variability in *D. pulex* resulting from the selection experiment. (*a*) Structure of the IGS repetitive region of 0.92 kb and 1.45 kb fragments. 28S, 18S and 5.8S boxes indicate rRNA genes; IGS, intergenic spacer region; ETS, external transcribed spacer; P, PCR primers. Individual repeats (shadowed boxes) are numbered according to their size in bp; each repeat contains a putative promoter sequence (black stripes). Sequences of each length variant may be found in GenBank (accession numbers AF456327 and AF456328 for 0.92 kb and 1.45 kb, respectively). (*b*) IGS length variants observed during the experiment on agarose gel (arrows with numbers indicate size variants in kb). (*c*) Frequency of IGS length variants (%, mean ± s.e.) in the fourth generation of the selected (H and L) and control (C) lines. The percentage of individuals with each type of banding pattern was calculated for each group of siblings originating from the same brood produced by third generation females (three females per line; altogether 30, 41 and 38 individuals analysed in L, H and C lines, respectively); error bars show variation between the three replicate lineages within each selection line. (White bars, single-band type; grey bars, double-band type.)

somatic growth rate and in body RNA and P contents. Higher RNA and P levels were observed in lines with higher JGRs and lower fecundity ('low WSF' selection line), while lower RNA and P levels were associated with higher fecundity ('high WSF' selection line). Moreover, increased frequencies of the longer IGS variants in the repetitive region, resulting from variation in the number of subrepeats, were positively correlated with JGR and body RNA and P contents as predicted by the growth rate hypothesis (Elser *et al.* 2000*a*). As a result of selecting on existing IGS heterogeneity, a new ('single-band') genotype was expressed in a significant proportion of the progeny of a female with two distinct types of IGS spacers. The lack of the 0.92 kb fragment in the majority of L line individuals in the fourth generation indicates that this line was on its way to fixation for the large IGS fragment through recombination during ameiotic reproduction. Thus, our data implicate rRNA genes in pleiotropic effects on life-history traits and show clear evidence of rapidly generated IGS length variation among individuals derived by parthenogenic reproduction from a single ancestral foundress.

Interestingly, by selecting for low WSF, we created a line that had accelerated somatic growth during the juvenile period, which resulted from faster maturation and earlier onset of reproduction. This result is consistent with previous observations on trade-offs between somatic growth and reproduction in various organisms (Stearns 1992), including *Daphnia* (Glazier 1999), and with the idea of antagonistic pleiotropy among genes affecting early and late life-history attributes (Rose & Charlesworth 1981). Why did only the low WSF selection regime show a consistent direct response? Two explanations are possible: the source population, owing to the long prior period of laboratory culturing, was pre-selected for high fecundity, inhibiting any further shift in this direction; or, alternatively, heritability of early fecundity (possibly leading to smaller clutches during the first adult instars) was greater than that of late fecundity, similar to results obtained for *Drosophila melanogaster* (Rose & Charlesworth 1981).

Apart from mutations, asexual progeny are believed to be genetically identical to their mothers (Vrijenhoek 1998). However, other studies have demonstrated rapid genetic changes within parthenogenic clones (reviewed by Lushai & Loxdale 2002). In aphids, for example, intraclonal genetic differences were observed after 5–14 generations (Lushai *et al.* 1998; Forneck *et al.* 2001). It is known that this variation is generated by various molecular mechanisms, including conventional DNA point mutations and errors of replication, slippage-mediated changes in hypervariable non-coding regions, such as IGS, and insertion and deletion of segments of DNA (see table 1 in Lushai & Loxdale 2002). Intraclonal variability of rDNA in *Daphnia* has been suggested (Crease & Lynch 1991). However, despite the apparent functional significance of variation in rDNA (Cluster *et al.* 1987; White & McLaren 2000), effects of rDNA variation on adaptive response in *Daphnia* have not been previously examined. The literature on relationships of growth and developmental rates to the structure of rDNA includes contradictory observations and hypotheses (Gray & Schnare 1996; Elser *et al.* 2000*a*). In copepods, White & McLaren (2000) found that developmental rates were positively related to 18S rDNA copy number per unit DNA, and suggested that levels of rDNA iteration have been controlled by natural selection to regulate ribosome concentration, protein production and growth. In *D. melanogaster* under selection for developmental rate, spacer length increased,

indicating that transcription rates of rDNA are increased by the presence of enhancer regions (Cluster *et al.* 1987). Similarly, in a long-term selection experiment in maize, selection for high grain yield was found to influence IGS length variation significantly (Rocheford *et al.* 1990). We found that longer spacers contain more copies of a 330 bp subrepeat, each containing an RNA polymerase promoter sequence, which has been suggested to enhance the rate of rDNA transcription. Thus, the advantage of long spacers may be manifested through greater rDNA transcriptional efficiency, ribosome production, protein synthesis and, hence, growth and development. Obviously, IGS length and structure are not the only factors influencing daphniid growth and developmental rates; however, together our results show a remarkable 'plasticity' of rDNA structure in clonal organisms and indicate that variation in spacer length, and possibly multiplicity of functional elements, is intrinsically linked to the growth phenotype of *D. pulex*. This may confer a selective advantage in heterogeneous environments, enabling mitotically reproducing lineages to evolve at least as fast as meiotic ones. Indeed, several lines of evidence indicate that *Daphnia* can evolve rapidly in response to environmental challenges (Tolline & Lynch 1994), so evolution via selection may often occur at a rate comparable or faster than that caused by stochastic demographic events (Fagerström *et al.* 1998).

Ecologically significant trade-offs are also likely to be associated with rapid-growth lifestyles (Arendt 1997). In aquatic ecosystems, zooplankton growth rate is critical for food-web structures/interactions and nutrient recycling because crustacean zooplankton are a main link between primary producers (phytoplankton) and higher consumers (fishes). At both the intra- and interspecific levels, heritable variation in body P levels associated with rapid growth of animals with long IGS may be manifested through organismal C : N : P stoichiometry. The positive correlation that we found between growth rate and RNA and P levels in juvenile *Daphnia* supports our hypothesis that rapidly growing organisms commonly have low C : P and N : P ratios. These low ratios reflect increased allocation to P-rich ribosomal RNA (Elser *et al.* 2000*a*), as rapid protein synthesis by ribosomes is required to support fast growth (Alberts *et al.* 1983) and RNA represents a biologically dominant pool of P in most organisms (Elser *et al.* 1996). Various studies suggest that, in turn, stoichiometric balance impinges on physiological properties, feeding behaviour, community organization, consumer population stability, trophic dynamics and, eventually, biogeochemical cycling in the ecosystem (Andersen 1997; Elser & Urabe 1999; Elser *et al.* 2000*a*). Indeed, such P-rich low N : P zooplankton taxa are more likely to experience food quality limitation due to inadequate P content of food (Sterner & Schulz 1998; Elser *et al.* 2000*b*). Such grazers are also inefficient recyclers of P in ecosystems: in an attempt to maintain physiological homeostasis, they sequester nutrients in their own biomass to different extents, and high sequestration corresponds to low recycling (Elser & Urabe 1999). However, a low degree of sequestration of P by taxa with high N : P increases its availability through rapid recycling, which is qualitatively similar to an increase in P supply rate.

It is clear that a combination of genetic and molecular mechanisms may be responsible for establishing the growth potential, rate of RNA synthesis and elemental composition of a parthenogenically reproducing organism. Our results strongly support the functional significance of rDNA IGS structure and its involvement in pleiotropic effects on life-history traits; they may also indicate a genetic mechanism for the evolution of these associations, and demonstrate that *Daphnia* (and perhaps other parthenogens) possess considerable potential for rapid adaptive change in major life-history traits. These relationships have major implications for understanding rates of evolution in clonal organisms, how selective advantage in heterogeneous environments creates evolutionary change and how evolutionary change impacts energy and material flow in food webs.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.