

## LETTER

# Genetically-based trade-offs in response to stoichiometric food quality influence competition in a keystone aquatic herbivore

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### Abstract

The genetic basis of organism response to stoichiometric mismatches between environmental availability and somatic demand is still poorly understood. This study reports a consistent genotype × environment interaction related to phosphorus : carbon availability to *Daphnia*. In multiple pairs of *Daphnia pulicaria* clones, genetic variation at the phosphoglucose isomerase (*Pgi*) locus indicated that *Pgi*-heterozygotes out competed *Pgi*-homozygotes under high P : C conditions, whereas the opposite outcome was observed under low P : C conditions. Estimates of phosphorus use efficiency indicated that homozygotes were significantly more efficient. However, homozygotes were comparatively less homeostatic. We hypothesize that lower specific activity of *Pgi* from homozygotes, which results in lowered energetic efficiency during the second glycolytic step, may underlie the competitive advantage enjoyed by homozygotes under low P : C (i.e. excess C) conditions. Our results show that analysing stoichiometric mismatches between diet and consumer should advance our quest for a fundamental understanding of the mechanisms driving genotype-environment interactions.

### Keywords

Allozymes, clonal competition, ecological stoichiometry, elemental homeostasis, nutrient limitation, phosphoglucose isomerase (*Pgi*).

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### INTRODUCTION

Genotype × environment interactions play a major role in the maintenance of genetic polymorphisms (Hedrick *et al.* 1976; Hedrick 1986), and are often explained by invoking ecological heterogeneity, and the tradeoffs associated with generalist strategies (Vrijenhoek 1978; Bell 1982). Studies on variants of central metabolic genes clearly demonstrate that balancing selection mediated by the environment underlies the maintenance of polymorphisms in diverse organisms (e.g. Cavaner & Clegg 1981; DiMichele & Powers 1982; Dykhuizen & Hartl 1983; Watt 1983; Carvalho 1988; reviewed in Eanes 1999; Watt & Dean 2000). Today, a major thrust is to understand how molecular properties of genetic variants interact with ecological variables to maintain polymorphisms (Feder & Mitchell-Olds 2003; Hedrick 2007).

Studies on the allozyme variants of the glycolytic enzyme, phosphoglucose isomerase (*Pgi*; EC 5.3.1.9), have contrib-

uted much to our understanding of balancing selection in diverse organisms (reviewed in Riddoch 1993). *Pgi* catalyses the reversible conversion of D-glucose-6-phosphate and D-fructose-6-phosphate in the second step of glycolysis (Nelson & Cox 2000). *Pgi* should keep the concentrations of intermediate metabolites as low as possible to attain highest energetic efficiency in glycolysis, because energy can be lost via spontaneous hydrolysis of energy storing phosphate groups (Watt 1985, 1986). *Pgi* is a dimer (comprised of two monomers), with homozygotes possessing a homodimer, while *Pgi* from heterozygotes is a heterodimer. Amino acid differences in key regions of component monomers affect the secondary structure of this enzyme resulting in significant differences (see Wheat *et al.* 2006 for a detailed treatment). *Pgi* from heterozygotes generally exhibits higher specific activity and lower thermal stability (Watt 1977), and these properties have explained, with remarkable accuracy, the distribution and thermal

ecology of genotypes in *Colias* butterfly populations (Watt 1977, 1983, 1983). *Pgi* is highly polymorphic in many organisms (e.g. *Escherichia coli*, Dykhuizen & Hartl 1983; *Colias* sp., Watt 1983; *Daphnia* sp., Geedey *et al.* 1996; Weider *et al.* 1997, 1999) and several studies show that the frequency of *Pgi* allozyme genotypes differ strongly depending on environmental factors (Riddoch 1993).

Temperature has been one of the most-studied environmental parameters shown to drive such balancing selection. Such fundamental physical parameters, and their subsequent role in driving microevolutionary change has been immensely useful in our quest to mechanistically understand evolutionary processes from genetic structure to individual fitness (Wheat *et al.* 2006; Orsini *et al.* 2009), and even dynamics at the metapopulation level (Hanski & Saccheri 2006).

Another common and fundamental ecological variable that is relevant to all organisms, and that is highly variable in space and time, is the availability of essential elements (e.g. carbon, phosphorus) (Sterner & Elser 2002). Stoichiometric theory is based on the observation that consumers have relatively fixed stoichiometry compared with the stoichiometry of the resources (i.e. food) they consume. As a result, consumers often encounter stoichiometric mismatches between their own demands and dietary supply, even in cases where there is an abundance of food (i.e. quantity, measured in terms of mass, calories or amount of carbon). Note that variation in stoichiometric resource quality (a ratio of two elements) results in two biochemically distinct challenges that the consumer should overcome for optimal performance: (1) to sequester the limiting element and (2) to process and either store or dispose the element in surplus. For example, *Daphnia* has a somatic phosphorus (P): carbon (C) ratio of *c.* 0.01 (i.e. one P atom per every 100 C atoms; Elser & Urabe 1999). When daphniids encounter algae with a P : C of *c.* 0.001 they are P-limited and can increase sequestration of P (e.g. by compensatory feeding; Plath & Boersma 2001), or find ways to dispose of excess C in food (Darchambeau *et al.* 2003; Jeyasingh 2007) or some combination of both. Consumers are frequently faced with diets that are in energetic excess (Raubenheimer & Simpson 1997; Sterner & Elser 2002). Under such situations, energetic efficiency of metabolic pathways may not translate into fitness payoffs.

Here, we explored the competitive performance (as a measure of fitness) of *Pgi* heterozygotes and homozygotes of *Daphnia pulicaria* under contrasting P : C dietary conditions in laboratory experiments. Furthermore, we quantified the phosphorus use efficiency (PUE), and the homeostatic coefficient (H,  $\eta$ ; Sterner & Elser 2002; see below for a detailed description) in terms of P : C, of these *Pgi* genotypes to identify potential drivers for competitive outcomes. While it is likely that the link between *Pgi*

genotype and P : C algal ratio, and its concomitant effects on survival, PUE, and H of genotypes is far more complex, laboratory simplification of the system to isolate ecological effects on specific genes should be useful to understand basic principles underlying such interactions (Dykhuizen & Dean 1990; Watt & Dean 2000).

## METHODS

### Experimental animals

Six *D. pulicaria* clones were isolated from Whitford (42°31' N, 85°35' W), Bristol (42°48' N, 85°24' W), or Long (42°54' N, 85°38' W) lakes near the Kellogg Biological Station, Michigan, USA. These clones were genotyped at *Pgi*, *Pgm* (phosphoglucomutase, EC 5.4.2.2), and *Ldb* (lactate dehydrogenase, EC 1.1.1.27) allozyme loci and, clonal isolates were maintained at 20 °C, and 18:6 light: dark cycle in low-phosphorus COMBO medium (Kilham *et al.* 1998), and fed *c.* 1 mg C<sup>-1</sup> L day<sup>-1</sup> of high phosphorus *Scenedesmus obliquus* algae. Experimental lineages thus established were paired, based on their *Pgi* genotype (i.e. fast homozygote or heterozygote), origin (same or different source lake) and *Pgm* genotype. By convention, faster electromorphs (i.e. alleles that migrated a greater anodal distance) were given a higher number designation than slower ones. The clones thus chosen were (with genotypes at '*Pgm*' and '*Pgi*' in parenthesis): Whitford-1 ('23' '04'), Whitford-2 ('22' '44'), Whitford-5 ('22' '04'), Whitford-8 ('22' '04'), Bristol ('22' '44') and Long ('22' '44'). Single mothers from chosen clones were propagated, and their great-grand-daughters (third generation) were used for experiments. All clones were scored as 'fast-fast' '33' homozygotes at *Ldb*, which confirmed that all clones were *D. pulicaria* (Hebert & Crease 1980). Henceforth, we denote clones that are heterozygotes at *Pgi* in CAPITAL letters to distinguish them from homozygotes (i.e. WHIT-1, WHIT-5, WHIT-8, and Whit-2, Bris, Long).

### Manipulation of dietary P : C

The green alga, *Scenedesmus obliquus*, was cultured in a continuous-flow chemostat kept inside a growth chamber at 20 °C and 24 h light. High phosphorus or low phosphorus medium (Kilham *et al.* 1998) were supplied to the algae to generate cells with contrasting P : C ratios. The concentration (mg carbon L<sup>-1</sup>) of the harvested algae was estimated spectrophotometrically using absorbance – C-content regressions, and its phosphorus content was estimated using the persulfate digestion method (APHA 1992). During the clonal competition experiments, *Scenedesmus obliquus* cells grown in high phosphorus medium had a phosphorus: carbon (P : C, molar) ratio of *c.* 0.01 (HP), and those grown in low phosphorus medium had a P : C *c.* 0.00125 (LP).

### Genotypic competition under different P : C conditions

Clonal competition experiments were conducted in 1L glass Mason jars. Each jar contained 1L of nitrogen and phosphorus-free COMBO medium (Kilham *et al.* 1998) and was stocked with twelve 1-day-old neonates of each clone (i.e. 1 : 1 initial proportion) in three experiments. *Experiment one*: Here, Whit-2 was in competition with WHIT-5 to test whether *Pgi* heterozygotes of *D. pulicaria* responded in the same way as *Daphnia pulex* counterparts under contrasting P-environments (i.e. heterosis under P-sufficiency; Jeyasingh & Weider 2005; Weider *et al.* 2005, 2008). *Experiment two*: To test whether similar competitive outcomes of different *Pgi* genotypes could be identified in clones isolated from different lakes, this experiment recorded the competitive interactions between Bristol (homozygote) and WHIT-8 (heterozygote). *Experiment three*: Finally, to test for the potential role of *Pgm* in determining competitive interactions of differing *Pgi* genotypes, this experiment analysed competitive interactions between Long (i.e. a double homozygote at *Pgm-Pgi*) and WHIT-1 (i.e. a double heterozygote at *Pgm-Pgi*).

Each treatment in each experiment was replicated five times, with one control jar per clone being maintained separately under both P-treatments. All jars were fed 3 mg carbon L<sup>-1</sup> of either HP or LP algae every 72 h (i.e. 1 mg C L<sup>-1</sup> day<sup>-1</sup>). Every 2 weeks (except for the second sample taken 3 weeks into the experiment), contents of each jar were gently homogenized using a glass rod, 100 mL was filtered through an 80 µm filter to collect daphniids, which were individually frozen at -20 °C in 96-well microtitre plates for subsequent allozyme screening (see below). The 100 mL filtrate was returned to each of the 1 L experimental jars. The experiments were terminated after 8 weeks. The number of daphniids in the 100 mL sample was used to estimate density (no. L<sup>-1</sup>) for the entire experimental jar (no. 100 mL<sup>-1</sup> × 10). Allozyme electrophoretic screening at the PGI-locus was done according to Hebert & Beaton (1993). Changes in genotypic frequencies within each competitive treatment were tested using repeated-measures ANOVA on the arcsine transformed proportions of *Pgi* genotypes present in each sample.

### Determination of PUE and H

We quantified PUE of each genotype after 5 days of experimental exposure to either high or low P diets. During the 5 days, HP cells had a P : C ratio of 0.009 ± 0.0004, and LP cells had 0.001 ± 0.00002. PUE is defined as the amount of biomass per unit of somatic P concentration. PUE (µg) =  $M/P_c$ , where,  $M$  = mass (µg),  $P_c$  = P concentration of biomass (µg µg<sup>-1</sup>). Neonates (< 24 h old) from each clone were measured to the nearest 0.02 mm (to

standardize initial size) and grown in batches of 20 in 200 mL jars containing N- and P-free medium (Kilham *et al.* 1998) and fed 1 mg C L<sup>-1</sup> of either high or low P algae daily to determine  $P_c$ . After 5 days, the animals were dried overnight at 60 °C, and weighed using a microbalance to the nearest 0.1 µg (to determine  $M$ ). Phosphorus content of animals was estimated using persulfate digestion and ascorbic acid method (APHA 1992) after ashing at 550 °C for 2 h. This experiment was replicated six times.

The degree of elemental homeostasis is expressed with the coefficient  $H$  ( $\eta$ ) (Sterner & Elser 2002). This coefficient is 1/slope where 'slope' comes from the linear fit of log-transformed variables of consumer nutrient content vs. resource nutrient content. In this case, means of the log P : C (molar) ratios were calculated for each clone under each of the two food treatments and the slope was calculated using P : C algal ratios given above. We determined C content of each clone from dry weight, assuming C is 48% of dry weight (Andersen & Hessen 1991).

## RESULTS

### Genotypic competition

*Daphnia* densities were higher in HP jars than in LP jars in all competition experiments. In control jars, observed *Daphnia* densities were lower in LP compared with HP in all six clones studied (statistical tests could not be performed Table 1). Similarly, the observed densities of all six clones were markedly different between control and experimental jars in both P : C treatments (Table 1). Repeated-measures ANOVA examining changes in the frequency of heterozygotes over the 63-day experimental period (five sampling times) in HP and LP treatments indicated strong phosphorus (food) treatment effects in all three experiments. In experiment one, P-treatment strongly affected heterozygote frequency in samples ( $F_{1,8} = 128.20$ ,  $P < 0.0001$ ; Fig. 1). A significant time × P-treatment interaction was found ( $F_{4,32} = 26.16$ ,  $P < 0.0001$ ). Data from experiment two also indicated a strong P-treatment effect ( $F_{1,8} = 205.23$ ,  $P < 0.0001$ ; Fig. 2), and time × P-treatment interaction ( $F_{4,32} = 10.37$ ,  $P < 0.0001$ ). Similarly, in experiment three, P-treatment significantly affected the frequency of heterozygotes ( $F_{1,8} = 38.33$ ,  $P = 0.0003$ ; Fig. 3) and there was a significant time × P-treatment interaction ( $F_{4,32} = 9.14$ ,  $P = 0.001$ ).

### PUE and H

Initial neonate size did not differ among clones (0.97 ± 0.04 mm;  $F_{5,66} = 1.43$ ,  $P = 0.22$ ). Dietary P : C had a major effect on PUE ( $F_{1,70} = 107.80$ ,  $P < 0.0001$ ; Fig. 4), because low phosphorus (LP) diet decreased PUE

**Table 1** Comparison of clonal densities in competition and control jars. Values are 99% confidence intervals for mean individuals L<sup>-1</sup>. Controls were not replicated but control densities of genotypes losing in competition lie well outside densities observed in competition conditions

	Experiment I		Experiment II		Control II		Experiment III		Control III	
	Whit2	WHIT5	Whit2	WHIT5	Bris	WHIT8	Bris	WHIT8	Long	WHIT1
P:C = 0.01										
Day 0	12 ± 0	12 ± 0	12	12	12 ± 0	12 ± 0	12	12 ± 0	12 ± 0	12 ± 0
Day 14	29.84–74.16	41.85–58.15	130	100	26.26–81.74	31.79–112.21	60	50	22.17–93.83	19.65–108.35
Day 21	25.27–102.73	18.86–89.14	130	40	31.2–104.8	53.86–118.14	100	140	17.02–46.98	33.03–74.97
Day 35	22.98–53.02	88.4–111.6	150	200	11.82–76.18	47.08–104.92	90	140	5.29–82.71	83.02–112.98
Day 49	5.98–34.11	126.97–157.03	140	190	18.52–53.48	140.98–167.62	160	200	5.08–62.92	105.03–146.97
Day 63	-1.63–17.63	149.31–206.69	210	140	-4.61–20.61	143.7–164.3	150	210	-6.3–14.3	134.38–181.62
P:C = 0.001										
Day 0	12 ± 0	12 ± 0	12	12	12 ± 0	12 ± 0	12	12	12 ± 0	12 ± 0
Day 14	11.79–48.21	14.73–53.27	80	110	24.92–59.08	27.02–56.98	110	100	7–57	10.07–49.93
Day 21	10.05–49.95	19.39–44.61	80	120	25.7–46.3	32.36–51.64	110	130	13.02–42.98	18.36–37.64
Day 35	25.2–98.8	16.98–47.02	40	70	15.39–40.61	3.76–36.24	110	110	30.15–77.85	4.76–43.24
Day 49	35.57–84.43	19.69–32.31	100	80	50.87–77.13	19.69–31.31	90	80	37.13–98.87	17.69–30.31
Day 63	27.48–80.52	0.54–31.46	60	120	47.39–88.61	11.8–48.2	40	50	51.8–88.2	-2.62–38.62

significantly. Within dietary treatments, PUE varied among genotypes only in LP conditions (HP;  $F_{5,30} = 0.94$ ,  $P = 0.46$ ; LP;  $F_{5,30} = 3.85$ ,  $P < 0.008$ ; Fig. 4). When clones were pooled based on their *Pgi* genotype, homozygotes had significantly higher PUE compared with heterozygotes ( $F_{1,4} = 7.94$ ,  $P = 0.04$ ).

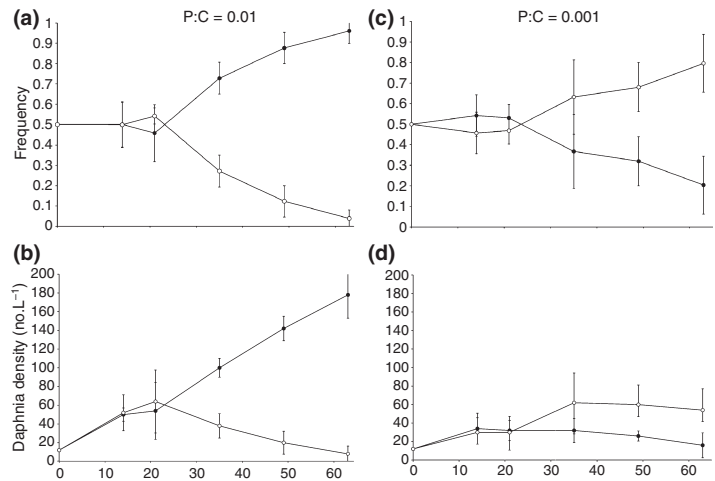
Finally, heterozygotes exhibited stronger P homeostasis than did homozygotes (Table 2). On average, heterozygotes had mean  $H = 83.59$  and homozygotes had mean  $H = 20.87$ . The  $H$ -values presented here are likely approximate in that only two relatively extreme endpoints of algal P content were tested, but the contrast in  $H$  observed here is relevant to these experimental conditions.

**DISCUSSION**

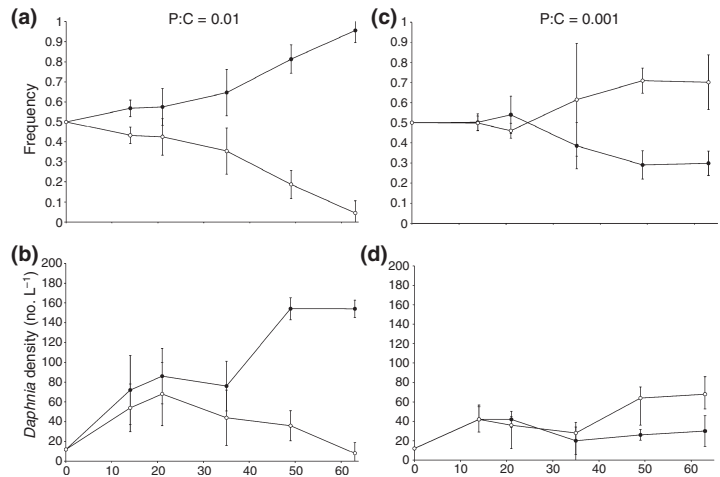
Competition clearly had a major effect on the densities of all six clones in both P : C treatments (Table 1). Competitive outcomes indicate a consistent *Pgi*-genotype × P : C-environment interaction in *D. pulicaria*. Heterozygotes are favored under high P conditions but homozygotes are favored under low P conditions. This same outcome was observed regardless of the origin of competing clones (Figs 1 and 2) or the *Pgm* genotype (Fig. 3). These results suggest that there is significant genetic variation in the responsiveness of daphniids to the P : C stoichiometry of their algal food (Jeyasingh & Weider 2007), and such responsiveness appears to be related to the *Pgi* genotype either directly or via linkage. We cannot exclude the possibility that competitive outcomes between these clones may be due to microevolutionary adaptation at other loci, and not due strictly to *Pgi* functionality. Clonal competition outcomes were consistent with the observed difference in PUE (Fig. 4), with *Pgi* homozygotes exhibiting significantly higher PUE under low P : C (LP) conditions. These differences also translated into a difference in homeostatic regulatory ability, with heterozygotes being more homeostatic with respect to their P content than homozygotes (Table 2).

Results from experiment one (Fig. 1) indicated a strong *Pgi*-genotype × P-environment interaction in two naturally coexisting *Pgi* clones of *D. pulicaria* (i.e. the homozygote, Whit-2 and the heterozygote WHIT-5), and were very similar to results reported by Weider *et al.* (2005) for competition experiments between two naturally coexisting *Pgi* genotypes of *D. pulex* (i.e. the heterozygous genotype outcompeted the homozygous genotype under HP conditions, while the reverse outcome was observed under LP conditions). Experiment two, where two clones (i.e. Bristol and WHIT-8) isolated from different lakes were allowed to compete resulted in a highly similar G × E interaction, possibly indicating that the relationship between the performance of various *Pgi* variants and the P-environment

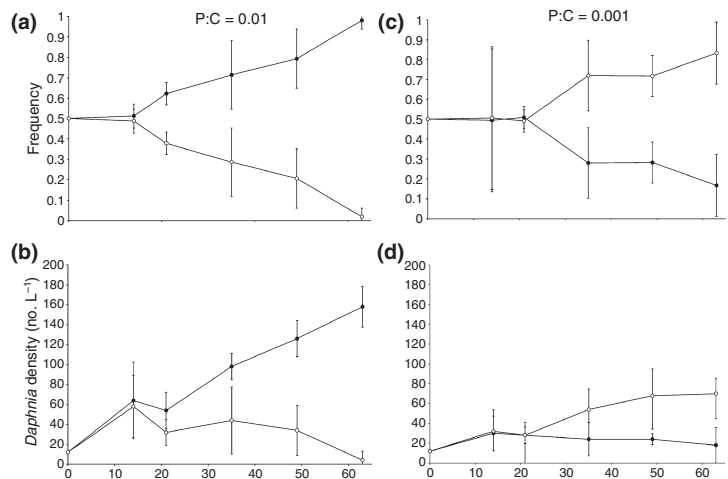
**Figure 1** Results of experiment one [Whit-2 (○); WHIT-5 (●)] showing (a) frequency ( $\pm 1$  SD) of homozygotes and heterozygotes in 100 mL samples (10% of total volume) and (b) showing the density (no.  $L^{-1} \pm 1$  SD) of homozygotes and heterozygotes over the 63-day experimental period under  $1 \text{ mg C L}^{-1} \text{ day}^{-1}$  of high phosphorus ( $P : C \approx 0.01$ ) algae. (c, d) Frequency and density of homozygotes and heterozygotes under low phosphorus ( $P : C \approx 0.001$ ) conditions, respectively.

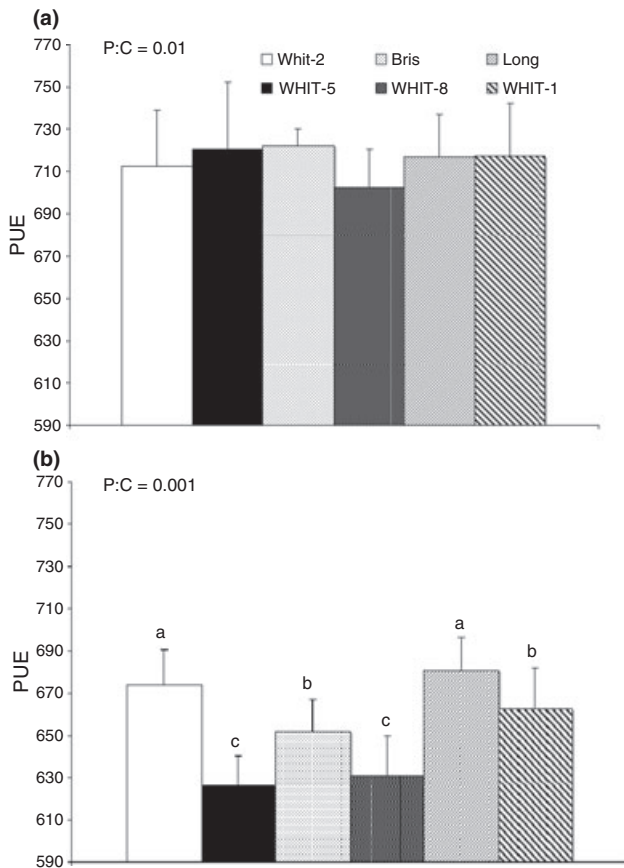


**Figure 2** Results of experiment two [Bristol (○); WHIT-8 (●)] showing (a) frequency ( $\pm 1$  SD) of homozygotes and heterozygotes in 100 mL samples (10% of total volume) and (b) showing the density (no.  $L^{-1} \pm 1$  SD) of homozygotes and heterozygotes over the 63-day experimental period under  $1 \text{ mg C L}^{-1} \text{ day}^{-1}$  of high phosphorus ( $P : C \approx 0.01$ ) algae. (c, d) Frequency and density of homozygotes and heterozygotes under low phosphorus ( $P : C \approx 0.001$ ) conditions, respectively.



**Figure 3** Results of experiment three [Long (○); WHIT-1 (●)] showing (a) frequency ( $\pm 1$  SD) of homozygotes and heterozygotes in 100 mL samples (10% of total volume) and (b) showing the density (no.  $L^{-1} \pm 1$  SD) of homozygotes and heterozygotes over the 63-day experimental period under  $1 \text{ mg C L}^{-1} \text{ day}^{-1}$  of high phosphorus ( $P : C \approx 0.01$ ) algae. (c, d) Frequency and density of homozygotes and heterozygotes under low phosphorus ( $P : C \approx 0.001$ ) conditions, respectively.





**Figure 4** Phosphorus use efficiency (PUE) of the six genotypes studied under (a) high phosphorus (P : C *c.* 0.01), and (b) low phosphorus (P : C *c.* 0.001) diets. Clones in paired competition are presented adjacent to each other, with darker bars representing heterozygotes. Error bars indicate  $\pm 1$  SD. Different letters indicate Tukey's *post hoc* significance.  $PUE (\mu\text{g}) = M/P_c$ , where,  $M$  = mass ( $\mu\text{g}$ ),  $P_c$  = P concentration of biomass ( $\mu\text{g } \mu\text{g}^{-1}$ ).

may have evolved earlier, and is common to at least closely-related *Daphnia* species. Finally, the possibility of such strong *Pgi*-genotype  $\times$  P-environment interactions being linked to another highly variable central metabolic enzyme, *Pgm* was tested in experiment three. Results indicate no such link; however, further systematic analyses using multiple genotypes in all combinations (i.e. heterozygotes and homozygotes varying at *Pgm* and *Pgi*) are required to establish whether *Pgm* affects the interaction between *Pgi* and the P-environment.

Results from the PUE experiments indicate that PUE declines in all genotypes under severe P-limitation (Fig. 4). Higher PUE of *Pgi* homozygotes under low P : C conditions in all three experiments (Fig. 4b) could contribute to their competitive advantage (Figs 1–3), although considerable variation in PUE exists within the *Pgi* genotypes studied. Interestingly, this intraspecific variation in PUE was

**Table 2** Degree of elemental homeostasis measured by the H coefficient (Sterner & Elser 2002) by clone. The difference between heterozygous and homozygotes was significant by nonparametric (Mann–Whitney *U*-test,  $P = 0.0495$ ) and by parametric (*t*-test,  $P = 0.0295$ ) tests

Clone	<i>Pgi</i> -genotype	Homeostasis coefficient (H, $\eta$ )
WHIT-1	Hetero	32.85
WHIT-5	Hetero	161.7
WHIT-8	Hetero	56.23
Bris	Homo	15.97
Long	Homo	25.22
Whit-2	Homo	21.42

not apparent under high P : C conditions (Fig. 4a). These results suggest that lower specific activity of *Pgi* from homozygotes, which results in loss of energy via spontaneous hydrolysis of phosphate groups in intermediary metabolites of glycolysis (Watt 1985, 1986), may in fact be advantageous under low P : C conditions where disposal of surplus C maybe at a premium. We acknowledge that metabolic networks are rarely so simplistic, and such claims cannot be validated until stringent metabolic flux analyses (e.g. Dauner *et al.* 2001) on *Pgi* variants are done.

Homeostatic regulatory ability is a key parameter in ecological stoichiometry (Sterner & Elser 2002). It quantifies the variation in the chemical content of a consumer relative to the chemical content of its resources. Stoichiometric models have often assumed a strict homeostasis ( $H = \infty$ ) for animals and a flexible stoichiometry for autotrophs, although this is recognized to be only an approximation appropriate for certain ecosystem-level models (Sterner 1990). The variation inherent to a strong but not strict homeostasis of animals may be highly relevant to their ecology and fitness. DeMott & Pape (2005) have shown strong interspecific heterogeneity in P homeostasis in *Daphnia*. They found  $H > 20$  for *Daphnia dentifera* and *Daphnia mendotae*. *Daphnia pulicaria* in their study had a relatively low (*c.* 8) H coefficient. The high, genotype-specific H coefficients observed for *D. pulicaria* in the present study suggest that there exists a much greater genetic variation for H than previously observed.

Growth (fitness) penalties of homeostasis are well understood. Consumers that maintain high P content for instance, are penalized under conditions of low P in their resources (Sterner & Elser 2002). Advantages to homeostasis have been more difficult to identify. The present study suggests an underlying genetic tradeoff that is relevant to this issue. The more elementally homeostatic heterozygotes were favoured under high-P food conditions but were disfavoured under poor food conditions. It is still unclear

what physiological or biochemical mechanism(s) might connect homeostasis and performance under high quality food. However, this study brings to light a relevant environmental correlate to the underlying fitness tradeoff that has resulted in a wide range in homeostatic regulation among organisms.

Recall that under low P : C conditions, *Daphnia* not only require more P, but also need to dispose of excess C (i.e. energy). The reversible isomerization of glucose-6-phosphate and fructose-6-phosphate catalysed by *Pgi* in glycolysis performs at highest energetic efficiency when intermediate substrates are kept at low levels, because energy is lost during spontaneous hydrolysis of phosphate groups (Watt 1986). Low levels of intermediary metabolites can be maintained by increasing enzyme specific activity. Boriss (2001) purified and characterized allozymes of *Pgi* from *Daphnia magna* and found that heterozygotes had a much higher specific activity compared with homozygotes. It could be possible that under high P : C diet (i.e. balanced diet), heterozygotes may have an advantage because of higher energetic efficiency in this critical step of glycolysis, while under low P : C diet (i.e. excess C), the energetic inefficiency caused by the lower specific activity of *Pgi* from homozygotes may in fact be advantageous. Several lines of evidence, in addition to this study, suggests a tight correlation between dietary P : C and the performance of *Pgi* genotypes in *Daphnia*, in the wild (Weider *et al.* 1997), and in controlled laboratory conditions (Jeyasingh & Weider 2005; Weider *et al.* 2005, 2008). It could be possible that lower efficiency of the second glycolytic step in *Pgi* homozygotes under low P : C conditions (i.e. excess C) confers a fitness advantage, while the higher efficiency of heterozygotes is selected for under high P : C conditions. Further exploration of the biochemical links between substrate P : C ratio, and *Pgi* structure is forthcoming.

Differential effects in response to dietary P : C may also be related to the strategic position of *Pgi* in central metabolism. *Pgi* action proceeds to glycolysis, whereas the alternative route leads to the pentose shunt pathway (Nelson & Cox 2000). Organisms are known to re-route resources into alternative metabolic pathways under P-limitation (Tillberg & Rowley 1989; Schachtman *et al.* 1998; Raghohama 2005), and even bypass phosphate-dependent reactions in glycolysis (Theodorou & Plaxton 1993). Furthermore, strong size-dependent respiratory responses to variation in P-content of algae were found in four species of daphniids (Jeyasingh 2007). Specifically, daphniids increased oxygen consumption when feeding on P-limited algae. However, increased respiration was not due to increased ingestion, and did not result in higher growth, indicating an uncoupling of oxygen consumption from ATP synthesis (i.e. diet-induced thermogenesis). The potential

role of *Pgi*, and its allelic variants in mediating such alternative pathways warrants further investigation, especially under low P : C conditions.

Finally, the multi-functionality of *Pgi*, which has at least three other distinct names based on its functions (Kao & Lee 2002): neuroleukin (*Nlk*), autocrine mobility factor (*Amf*), and differential action and maturation mediator (*Dmm*), suggests that eco-evolutionary studies on *Pgi* should consider all other functions of *Pgi*, in addition to its role in glycolysis. Results from this study clearly indicate strong *Pgi*-linked G × E under laboratory conditions where we controlled for other environmental variables, including temperature. Furthermore, the stoichiometric approach discussed here is readily amenable to analyses of potential molecular mechanisms underlying observed G × E interactions. Such analyses of interactions between key niche axes (e.g. nutrient supply) and central metabolic genes (e.g. *Pgi*) should be ideal systems to understand the biochemical, and physiological mechanisms underlying G × E interactions. Finally, these results suggest that dietary stoichiometry is an important environmental parameter influencing competitive interactions and (ultimately) microevolutionary trajectories of genotypes.

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