Allozymes of glucose-6-phosphate isomerase differentially modulate pentose-shunt metabolism in the sea anemone *Metridium senile*

(enzyme polymorphism/metabolic flux/temperature adaptation/natural selection/Cnidaria)

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ABSTRACT We tested the hypothesis that kinetic differences among allelic variants of glucose-6-phosphate isomerase (GPI; D-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9) from the sea anemone *Metridium senile* differentially modulate glucose metabolism at the glycolysis–pentose-shunt branch point. Fractional contribution of pentose shunt and absolute flux of glucose in glycolysis were measured in fasted or fed anemones acclimated to 5°C or 15°C. When fed, anemones of genotype *Gpi^{ss}* routed a greater fraction of glucose through the shunt than did *Gpi^{ff}* anemones; the effect was more pronounced at 5°C than at 15°C. This confirms predictions from kinetic and population data and is consistent with thermal selection maintaining the variation. Relative levels of shunt metabolism increased at 5°C, compared with 15°C, in fed anemones regardless of genotype, but the proportion of glucose metabolized by the pentose shunt was unchanged by temperature in fasted anemones. Glucose flux through the shunt was constant at approximately 5 pmol·mg⁻¹·hr⁻¹ in fed anemones at 5°C and 15°C and in fasted anemones at 15°C, indicating apparently near-perfect thermal acclimation of the absolute flux of glucose through the shunt in fed, but not in fasted, anemones. Rates of glucose oxidation and flux through the shunt in freshly collected anemones were similar to those of anemones fed and acclimated at 15°C in the laboratory. If these differences affect organismal-level processes, *Gpi* variation could contribute to Darwinian fitness in thermally varying environments.

A central issue in evolutionary biology is the elucidation of mechanisms by which genetic variation might lead to differences in fitness among individuals in a population. Many studies have shown on the basis of *in vitro* kinetics that functional differences may exist among allelic enzyme variants (allozymes) (1–8). In several important cases, this biochemical approach has been extended to include organismal-level correlates of genetic variation. Physiological and behavioral differences among individuals have been accurately predicted from kinetic differences among enzyme variants, and persuasive arguments have been made that natural selection acts ultimately upon organismal-level differences deriving from kinetic differences among allozymes (9–15).

Recent theoretical considerations suggest that variation in properties of any single enzyme in a pathway may have little effect on pathway flux (16, 17), a view supported by work with alcohol dehydrogenase mutants in *Drosophila melanogaster* (18). Nonetheless, a few studies show that allozyme variation may influence metabolism. For example, variants of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase alter pentose-shunt metabolism in *Drosophila* (19), and rates of alanine accumulation during osmotic stress are influenced by activity variants of glutamic-

pyruvic transaminase (alanine aminotransferase) in a copepod, *Tigriopus californicus* (20). Preliminary data suggest that allozymes of glucose-6-phosphate isomerase (GPI; D-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9) influence glycolytic flux in *Colias* butterflies (21). To establish that kinetic differences have the potential to lead to fitness-related differences among individuals, such predictable alterations in activities of the pathways of intermediary metabolism that arise directly from kinetic differences among the gene products of alternative alleles must be demonstrated. These demonstrations tighten the causal link between allozyme variation and Darwinian fitness.

We have directly measured the effects of allelic variation for GPI on glucose flux through the glycolysis–pentose-shunt branch point in a sea anemone, *Metridium senile* (L.), by using D-[3-³H]glucose in combination with either D-[1-¹⁴C]glucose or D-[6-¹⁴C]glucose (22–24) to test the hypothesis that *Gpi* variation contributes to temperature adaptation through its effects on glucose metabolism. Populations of *M. senile* from the northeast coast of the U.S. are polymorphic for the glucose-6-phosphate isomerase gene (*Gpi*), which has two alleles, designated *Gpi^f* and *Gpi^s* according to the electrophoretic mobility of their products (25, 26). *Gpi* allele frequencies vary clinally, paralleling a steep thermal gradient (25), and the allozymes differ in their kinetic properties. Of special interest, the *ss* allozyme is more inhibited by a pentose-shunt intermediate, 6-phosphogluconate, than is the *ff* allozyme, especially at low temperatures. An allozyme that is more strongly inhibited by a pentose-shunt intermediate might more strongly redirect carbon away from glycolysis and into the pentose-shunt pathway, particularly at colder temperatures. Until now, support for the hypothesis that *Gpi^{ss}* anemones might process a greater fraction of metabolized glucose through the shunt than *Gpi^{ff}* anemones (2, 3), especially at cold temperatures, has rested on the biogeography of allele frequency variation; the frequency of the *Gpi^s* allele increases with declining water temperature to the north of Cape Cod, suggesting that the kinetic differences may contribute to thermal adaptation. On the basis of *in vitro* kinetic data, we expected that fractional contribution of the shunt would be higher in *Gpi^{ss}* anemones compared with *Gpi^{ff}* anemones, an effect that might be most pronounced at colder temperatures. We demonstrate that temperature and *Gpi* genotype modulate pentose-shunt metabolism as suggested by the kinetic and population data.

MATERIALS AND METHODS

Collection and Acclimation of Anemones. Individuals of *M. senile* (approximate mean of wet mass, 350–400 mg) were collected from the underside of the public dock at Bartlett's

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Abbreviations: GPI, glucose-6-phosphate isomerase; *Gpi*, gene for GPI.

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Landing (Mount Desert Island, ME) by divers at various times during 1987–1988. Anemones used in "fed" and "fasted" experimental series were shipped by air to Ames, where they were held in artificial seawater at 15°C. Subsequently, small pieces of tissue were removed from basal discs and used to determine the genotype of anemones at five polymorphic loci (*Gpi*, two loci encoding leucine aminopeptidase, and loci for phosphoglucosyltransferase and octopine dehydrogenase) by starch gel electrophoresis (2, 25, 27). Genotyped anemones were placed in individual containers at 5°C or 15°C in recirculating seawater (Instant Ocean Aquarium Systems, Eastlake, OH; 30–33 parts per thousand salinity). Fasted anemones were starved for 5–7 weeks, and fed anemones were provided with *Artemia* sp. nauplii *ad libitum* every second day during 3–5 weeks of acclimation. In the final series of experiments (field), which were designed to assess the influence of natural diet, anemones were collected from Bartlett's Landing in June 1988 (ambient water temperature, 11°–16°C) and transported to Mount Desert Island Biological Laboratory. Field anemones were screened for *Gpi* genotype by using cellulose acetate electrophoresis (Helena Laboratories, 0.025 M Tris/0.19 M glycine buffer) and placed in individual containers in flowing seawater. A second piece of tissue was removed and frozen for later genotyping at the four remaining loci with starch gels. Elapsed time between the collection of field anemones and initiation of incubation in isotopes ranged from 4 to 6 hr.

Because *M. senile* reproduces asexually, anemones with unique five-locus genotypes were used in the fasted and fed experimental series to ensure that effects of GPI on the pentose shunt could be assessed against randomized genetic backgrounds. This was not possible in the field experiments because anemone tissues were used before genotyping at all five loci was complete. Results of GPI effects in the field experiments could be biased by the use of clonemates.

Carbon-Flux Measurements. Pentose-shunt metabolism was assessed by measurement of $^{14}\text{CO}_2$ and tritiated water production by anemone tissues (22, 23). $^{14}\text{CO}_2$ is produced by oxidation of [6- ^{14}C]glucose in glycolysis and the tricarboxylic acid cycle or from the catabolism of [1- ^{14}C]glucose in the pentose shunt in addition to oxidation in glycolysis and the tricarboxylic acid cycle. One molecule of tritiated water is formed for each molecule of D-[5- ^3H]glucose converted to pyruvate or phosphoenolpyruvate, providing an estimate of glycolytic flux (23). Rates of glycolysis [= utilization of Ashcroft *et al.* (22)] are higher than the sum of glucose oxidation from [1- ^{14}C] and [6- ^{14}C]glucose because a fraction of pyruvate formed in glycolysis is not metabolized in the tricarboxylic acid cycle and therefore, may not result in CO_2 production.

Four radial sections from each anemone were blotted and weighed. Duplicate sections were incubated in a mixture of 1 μCi (37 kBq) of [5- ^3H]glucose and 1.5 μCi of either [1- ^{14}C] or [6- ^{14}C]glucose (Amersham) in 4 ml of filtered sea water (0.45 μm ; 33 parts per thousand salinity) containing 1 mM glucose. Specific radioactivities of [5- ^3H]glucose (1.04 dpm/pmol of glucose) and of [1- ^{14}C] and [6- ^{14}C]glucose (1.64 dpm/pmol of glucose) were determined by scintillation counting. The final glucose concentration was measured spectrophotometrically with a linked-enzyme assay including hexokinase (EC 2.7.1.1) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49). After tissue slices were placed in 25-ml flasks containing incubation medium, the flasks were sealed with rubber stoppers. Duplicate control flasks (without tissue) were prepared for each [^{14}C]glucose compound. Flasks were shaken for 6 hr in a water bath at the acclimation temperature (5°C and 15°C for fed and fasted anemones and 15°C for field anemones). Incubation was terminated by injection of 1 ml of 2 M perchloric acid into the flasks. Shaking continued for another 2 hr to trap $^{14}\text{CO}_2$ in hyamine

hydroxide (300 μl) contained in plastic cups suspended from the stoppers. Production of $^{14}\text{CO}_2$ was measured by counting radioactivity in the cups. Tritiated water was evaporated at 35°C for 48 hr from a 400- μl aliquot of the incubation medium over 400 mg of anhydrous CaCl_2 . The CaCl_2 was dissolved in deionized water. All samples were counted in 10 ml of Scintiverse II (Fisher).

Calculations and Statistical Analyses. Because significant negative regressions of the logarithm of radioactivity (dpm) vs. the logarithm of tissue section mass were obtained for $^{14}\text{CO}_2$ and tritiated water production, metabolic rates were corrected for control values and adjusted to a common mass of 90.6 mg (the mean of sections from all experiments) by covariance analysis. Oxidation (from $^{14}\text{CO}_2$ production) and glycolytic flux (from tritiated water production) for each anemone were calculated as means of two replicates (each for [1- ^{14}C] and [6- ^{14}C]glucose) and four replicates (for [5- ^3H]glucose). The proportion of glucose metabolism that occurred in the pentose shunt was calculated from the rates of $^{14}\text{CO}_2$ and tritiated water production by using equation 3 of Katz and Wood (24). Statistical tests were run on angular-transformed proportions (28). The flux of glucose through the pentose shunt was calculated by multiplying glycolytic flux by the proportion of glucose metabolized by the shunt (22, 23).

Statistical analysis was performed by using the general linear models of the Statistical Analysis System (SAS Institute; Cary, NC). A split-plot analysis of variance was used in which temperature (5°C or 15°C) was assigned to specific days. Numbers of anemones of each *Gpi* genotype (three each of *ff* and *fs*; two of *ss*) were fixed for each experiment to ensure a proportional design for increased statistical power, but measurements were made blindly with respect to *Gpi* genotype. The null hypothesis—that shunt metabolism was equivalent among *Gpi* genotypes—was conservatively tested by using an error mean square describing the interaction of *Gpi* genotype variation with random, daily variation of shunt metabolism within each temperature level (fasted and fed experiments). The main-treatment effect of temperature was tested by using an error mean square describing random, daily variation of shunt metabolism within each temperature level. In field experiments (all performed at 15°C), a mixed model analysis of variance accounting for random daily variation and main-treatment effects of *Gpi* genotype was used. Preplanned pairwise comparisons were made by using least-squares means from the general linear-models procedure. Because kinetic data (2) led us to predict that *Gpi^{ss}* anemones should direct a greater fraction of glucose to the pentose shunt than *Gpi^{ff}*, one-tailed tests were used to compare fractional contributions of the shunt in these two genotypes. Inhibition kinetics are not available for heterozygotes, so two-tailed tests were used in comparisons involving *Gpi^{fs}*.

RESULTS

The proportional contribution of the pentose shunt was significantly influenced by *Gpi* genotype of fed anemones when both temperatures were considered together ($P = 0.035$). In pairwise comparisons, the fractional contribution of the pentose shunt in fed anemones was significantly smaller in *Gpi^{ff}* anemones than in either *Gpi^{fs}* ($P = 0.0222$) or *Gpi^{ss}* anemones ($P = 0.0144$) regardless of temperature. Furthermore, this ordering of values was most apparent at 5°C (Fig. 1), where *Gpi^{ff}* anemones directed a significantly smaller fraction (by 28%) of glucose to pentose-shunt metabolism than did *Gpi^{ss}* anemones ($P = 0.0364$); a similar pattern was seen at 15°C for *Gpi^{ff}* vs. *Gpi^{fs}* anemones ($P = 0.0241$; Fig. 1). Significant interactions of temperature and *Gpi* genotype on the relative levels of pentose-shunt metab-

FIG. 1. Fractional contribution of the pentose-shunt pathway in *M. senile*. (A) Anemones acclimated and measured at 5°C. (B) Anemones acclimated at 15°C or freshly collected from the field and measured at 15°C. Error bars represent 1 SEM. Bars: □, fasted anemones (laboratory); ▨, fed anemones (laboratory); ■, field anemones (freshly collected).

olism were not observed ($P > 0.05$). This modulation by *Gpi* of the fractional contribution of the pentose shunt occurred even though *Gpi* did not significantly affect oxidation of either [1-¹⁴C]- or [6-¹⁴C]glucose or flux of glucose in glycolysis in fed anemones (Table 1). Moreover, absolute flux through the pentose shunt was equivalent among fed anemones of all *Gpi* genotypes (Table 1).

Comparisons of means showed that lowered temperature in fed anemone experiments significantly increased the fractional contribution of the pentose shunt in anemones of all genotypes (*ff*, $P = 0.0002$; *fs*, $P = 0.0026$; *ss*, $P = 0.0004$; Fig. 1). Likewise, significant reductions were observed in rates of glucose oxidation and glycolytic flux at 5°C compared with 15°C (Table 1). However, absolute flux of glucose in the pentose shunt in fed anemones was unaffected by temperature (Table 1). Thus, the thermal response of absolute glucose flux through the shunt is different than the proportional

response to temperature in fed (and fasted; see below) anemones. An apparent metabolic reorganization occurred in fed anemones between 15°C and 5°C in which oxidation of [6-¹⁴C]glucose was reduced disproportionately compared to smaller, equivalent reductions in glycolysis and oxidation of [1-¹⁴C]glucose.

In experiments with fasted anemones, no effect of *Gpi* genotype was seen on any of the measured components of glucose metabolism (Table 2). Lowered temperature significantly reduced rates of both glucose oxidation and glycolysis and of glucose flux through the pentose shunt (Table 2). However, the fractional contribution of the pentose shunt did not change with temperature (Fig. 1; mean of all fasted groups = 2.5%).

In field experiments, no genotypic effect was seen on glycolytic flux, glucose flux through the pentose shunt, or fractional contributions of the shunt (Table 3; Fig. 1). However, *Gpi* genotype significantly altered oxidation of [6-¹⁴C]glucose and tended to do the same for [1-¹⁴C]glucose (Table 3). Pairwise comparisons of means indicated that rates of both [6-¹⁴C]- and [1-¹⁴C]glucose oxidation were higher in *Gpi^{ff}* than in *Gpi^{ss}* anemones ($P = 0.0112$ and 0.0366 , respectively). Lastly, rates of glycolysis were substantially higher in field anemones than in either fasted or fed anemones (Tables 1–3).

DISCUSSION

Allozymes of GPI significantly altered the fractional contribution of the pentose-shunt pathway in laboratory-fed individuals of *M. senile*. In particular, *Gpi^{ss}* anemones more strongly redirected glucose to the shunt than did *Gpi^{ff}* anemones. When the effects of temperature are considered, the ordering of genotypes is most apparent at 5°C, where the fractional contribution of the pentose shunt to carbohydrate metabolism in *Gpi^{ff}* anemones was only 78% of that in *Gpi^{ss}* anemones. These results are in agreement with our expectations based on previous kinetic studies, which showed that the *Gpi^{ss}* allozyme is more inhibited by 6-phosphogluconate, a pentose-shunt intermediate, than is the *Gpi^{ff}* allozyme (2). Our attribution of these effects to the *Gpi* genotype is strengthened by knowledge that other enzymes surrounding the glucose 6-phosphate branch point either lack electrophoretic variants (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase; unpublished data) or have

Table 1. Pentose-shunt metabolism in fed anemones of each *Gpi* genotype acclimated to 5°C and 15°C

	Oxidation* (from ¹⁴ CO ₂ production)		Utilization* [from ³ H ₂ O production (glycolytic flux)]	Pentose-shunt flux*
	[6- ¹⁴ C]Glucose	[1- ¹⁴ C]Glucose		
Results at 5°C for genotype				
<i>ff</i>	4.61 ± 0.32	16.08 ± 1.24	91.04 ± 4.97	4.57 ± 0.58
<i>fs</i>	4.67 ± 0.32	17.43 ± 1.22	88.43 ± 4.75	5.12 ± 0.52
<i>ss</i>	5.47 ± 0.34	18.06 ± 1.00	81.67 ± 6.76	5.21 ± 0.57
Results at 15°C for genotype				
<i>ff</i>	24.03 ± 1.60	34.86 ± 3.34	176.29 ± 9.83	5.06 ± 1.38 [†]
<i>fs</i>	23.07 ± 1.78	37.26 ± 3.90	169.60 ± 8.27	6.72 ± 2.09 [†]
<i>ss</i>	24.99 ± 3.13	38.86 ± 3.67	182.69 ± 8.84	5.72 ± 1.13
Analysis of variance				
Temp.	<i>P</i> = 0.0004	<i>P</i> = 0.0017	<i>P</i> = 0.0001	<i>P</i> = 0.6816
<i>Gpi</i>	<i>P</i> = 0.4099	<i>P</i> = 0.2852	<i>P</i> = 0.7730	<i>P</i> = 0.1820
Temp. × <i>Gpi</i>	<i>P</i> = 0.8007	<i>P</i> = 0.8615	<i>P</i> = 0.4021	<i>P</i> = 0.5004

*Units are pmol of glucose·mg⁻¹·hr⁻¹; values are means ± SEM for 12 *Gpi^{ff}* anemones, 12 *Gpi^{fs}* anemones, and 8 *Gpi^{ss}* anemones except as indicated.

[†]No. of anemones examined was 11.

Table 2. Pentose-shunt metabolism in fasted anemones of each *Gpi* genotype acclimated to 5°C and 15°C

	<i>n</i>	Oxidation* (from ¹⁴ CO ₂ production)		Utilization* [from ³ H ₂ O production (glycolytic flux)]	Pentose-shunt flux*
		[6- ¹⁴ C]Glucose	[1- ¹⁴ C]Glucose		
Results at 5°C for genotype					
<i>ff</i>	9	5.02 ± 0.65	12.90 ± 0.70	114.11 ± 5.36	2.89 ± 0.18
<i>fs</i>	8	4.54 ± 0.36	13.39 ± 0.67	128.14 ± 5.60	3.22 ± 0.28
<i>ss</i>	6	4.42 ± 0.56	11.77 ± 0.45	112.24 ± 6.37	2.67 ± 0.18
Results at 15°C for genotype					
<i>ff</i>	6	23.15 ± 2.08	36.49 ± 1.97	211.42 ± 8.93	5.24 ± 0.63
<i>fs</i>	5	24.58 ± 1.61	36.80 ± 2.26	222.22 ± 2.34	4.79 ± 0.81
<i>ss</i>	4	23.88 ± 1.40	37.38 ± 2.77	205.27 ± 8.62	5.43 ± 0.97
Analysis of variance					
Temp.		<i>P</i> = 0.0031	<i>P</i> = 0.0009	<i>P</i> = 0.0003	<i>P</i> = 0.0020
<i>Gpi</i>		<i>P</i> = 0.4979	<i>P</i> = 0.5620	<i>P</i> = 0.1621	<i>P</i> = 0.9890
Temp × <i>Gpi</i>		<i>P</i> = 0.2384	<i>P</i> = 0.4315	<i>P</i> = 0.9541	<i>P</i> = 0.5164

*Units are pmol of glucose·mg⁻¹·hr⁻¹; values are means ± SEM (*n* = number of anemones).

kinetically equivalent allozymes, as does phosphoglucomutase (29).

Numerous studies have correlated functional differences among GPI allozymes with aspects of thermal adaptation and/or the potential for differential modulation of pentose-shunt metabolism (1–4, 14, 30). The present results provide direct confirmation of the modulatory role of functional variants of GPI. It is important to note, however, that the modulatory effect of *Gpi* genotype was observed for relative levels of pentose-shunt metabolism in fed anemones, not in fasted ones. In a variety of organisms, pentose-shunt metabolism decreases during fasting and is altered by the quality of the diet, particularly carbohydrate and lipid content (31–33). More importantly, activity differences in pentose-shunt metabolism among allozymes of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are accentuated under high-sucrose diets in *D. melanogaster* (19). Therefore, nutritional status is a strong determinant of the modulatory role played by associated allozymes on pentose-shunt metabolism in both *D. melanogaster* and *M. senile*.

The significant effect of genotype on the fractional contribution of pentose shunt, without corresponding statistically significant effects on glycolytic flux or pentose-shunt flux at first seems puzzling. However, it is critical to note that the method used (22, 24) yields only two of these metabolic quantities: glycolytic flux and the proportion of carbohydrate metabolism accounted for by pentose shunt. Shunt flux is calculated as the product of these values. It may not be surprising that multiplying a quantity showing significant effects of genotype (proportion of shunt metabolism) by another that does not show significant genotypic effects

produces values that do not show significant effects. There are joint countervailing trends among genotypes in glycolytic flux and pentose-shunt flux that are consistent with our hypothesis. For example, at 5°C in fed *Gpi^{ss}* anemones, glycolytic flux averages 90% and pentose-shunt flux averages 114% of the corresponding values in *Gpi^{ff}* anemones. It is important that these ordered trends are accompanied by the significant ordered pattern in proportion of shunt metabolism that is in the predicted direction.

Not only is nutritional history an important consideration in elucidating genetic modulation of pentose-shunt metabolism in *M. senile*, it also plays a pivotal role in determining the pattern of metabolic reorganizations that accompany thermal adaptation in this species. The present results conditionally confirm the increased relative importance of pentose-shunt metabolism in poikilotherms at colder temperatures that has been described in other studies in which glucose utilization was not measured (34, 35). However, increased fractional contribution of the pentose shunt between 15°C and 5°C was observed in fed anemones only, not in fasted ones. In fasted anemones, the relative level of shunt metabolism remained the same between 15°C and 5°C, and shunt flux declined with acclimation temperature by an average of 43%. In contrast, the metabolic reorganization of the relative levels of pentose-shunt metabolism at 5°C compared to 15°C in fed anemones results in stabilization of shunt flux at approximately 5 pmol of glucose·mg⁻¹·hr⁻¹ regardless of temperature. Therefore, pentose-shunt flux exhibits apparently near-perfect thermal acclimation between 5°C and 15°C in fed anemones, but not in fasted ones. Thus, temperature and nutritional history interactively alter glucose flux through the pentose shunt

Table 3. Pentose-shunt metabolism in field anemones of each *Gpi* genotype

	Oxidation* (from ¹⁴ CO ₂ production)		Utilization* [from ³ H ₂ O production (glycolytic flux)]	Pentose-shunt flux*
	[6- ¹⁴ C]Glucose	[1- ¹⁴ C]Glucose		
Results for genotypes				
<i>ff</i>	26.13 ± 1.85	38.00 ± 1.77	330.04 ± 11.25	5.22 ± 0.47
<i>fs</i>	22.84 ± 1.17	36.59 ± 1.46	319.20 ± 8.20	5.14 ± 0.54
<i>ss</i>	18.88 ± 1.68	31.68 ± 2.14	304.02 ± 9.03	4.73 ± 0.71
Analysis of variance				
<i>Gpi</i>	<i>P</i> = 0.0340	<i>P</i> = 0.0925	<i>P</i> = 0.2560	<i>P</i> = 0.7013

*Units are pmol of glucose·mg⁻¹·hr⁻¹; values are means ± SEM of 12 *Gpi^{ss}* anemones, 18 (oxidation) or 17 (utilization and pentose-shunt flux) *Gpi^{fs}* anemones, and 18 (oxidation) or 16 (utilization and pentose-shunt flux) *Gpi^{ff}* anemones.

independently of glucose flux in glycolysis, which declines with acclimation temperature by approximately 50% in both fed and fasted anemones. The similar flux levels in field, fed, and fasted anemones at 15°C indicate the independence of glucose flux through the pentose shunt from the nutritional history at this temperature only and validate the relevance of the laboratory studies to habitat conditions. Although few examples are available in which glucose flux in the pentose shunt has been measured in poikilotherms (36, 37), estimates of flux through the pentose shunt are similar in mantle tissues from fed and fasted blue mussels, *Mytilus edulis* (37), measured at 20°C. Further study is required to determine whether the metabolic organization observed at 5°C in laboratory-fed anemones is indicative of their metabolism *in situ* at 5°C. Additionally, differences in glycolytic flux between laboratory-held and field anemones, which probably are due to qualitative differences between artificial and natural diets, warrant further study.

The importance of GPI modulation of pentose-shunt metabolism in fed anemones to Darwinian fitness cannot be addressed directly at this time. However, the pentose shunt generates reduced cofactor (NADPH), required for lipid synthesis, and lipid synthesis rates have been linked to pentose-shunt activity in a number of systems (19, 31–33). Furthermore, allozymes of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase modulate lipid synthesis in *D. melanogaster* apparently through modifications of shunt activity (19). Lipid is required for the production of gonadal tissues in *M. senile*, and gonad development may occur at colder temperatures in the northern part of the geographic range of this anemone, where frequency of the *Gpi^s* allele increases.

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