Genetically Determined Variation in Developmental Physiology of Bivalve Larvae (*Crassostrea gigas*)*

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ABSTRACT

Understanding the complex interactions that regulate growth and form is a central question in developmental physiology. We used experimental crosses of pedigreed lines of the Pacific oyster, *Crassostrea gigas*, to investigate genetically determined variations in larval growth and nutrient transport. We show that (i) transport rates at 10 and 100 μM glycine scale differentially with size; (ii) size-specific maximum transport capacity (J_{max}) is genetically determined; and (iii) J_{max} serves as an early predictive index of subsequent growth rate. This relationship between genetically determined J_{max} and growth suggests the potential use of transporter genes as biomarkers of growth potential. Analysis of the genome of *C. gigas* revealed 23 putative amino acid transporter genes. The complexity of gene families that underpin physiological traits has additional precedents in this species and others and warrants caution in the use of gene expression as a biomarker for physiological state. Direct in vivo measurements of physiological processes using species with defined genotypes are required to understand genetically determined variance of nutrient flux and other processes that regulate development and growth.

Introduction

During development many animals have high growth and mortality rates. Variability in these traits impacts species fitness and adult recruitment success at the population level. Growth and development are affected by environment and genetics, as well as the interaction of these components. Variance in biological responses can be classified into genetic ("nature") and environmentally determined ("nurture") components. While the effects of environment on growth and development have been well characterized (Gilbert and Epel 2009), genetically determined variations in developmental physiology are less well understood. Genetically based experimental model organisms, such as fruit flies and other organisms with advanced genetics, have revolutionized our understanding of developmental biology (Davidson 2006; Gilbert 2013). Similar models allowing the study of genotype-dependent physiological functions in marine animals are lacking. We have developed pedigreed lines of the Pacific oyster, *Crassostrea gigas*, to repeatably produce larval families with genetically determined contrasts in growth and physiology (Hedgecock et al. 1995; Bayne et al. 1999; Pace et al. 2006; Hedgecock and Davis 2007; Hedgecock et al. 2007; Curole et al. 2010; Meyer and Manahan 2010). This approach has enabled a mechanistic understanding of genetic components underpinning variance in developmental physiology of the species.

One of the potential physiological mechanisms that can contribute to differential growth is variation in the ability of larvae to obtain nutrients from the environment. For instance, genotype determines major differences in algal feeding rates by larvae of *C. gigas* (Pace et al. 2006). The goal of the current study is to ascertain the significance of genotype in determining differences in the ability of larvae to transport dissolved organic nutrients from seawater. All phyla of marine invertebrates, with the exception of arthropods, are capable of absorbing dissolved organic material (DOM) across the body wall from low concentrations found in natural seawater (Jørgensen 1976; Stephens 1988; Wright and Manahan 1989). Marine DOM is a complex and variable mix of molecules (Benner 2002) that are difficult to identify and have chemical species with unknown biological functions. Fractions of the DOM pool, such as dissolved free amino acids, are frequently used to study nutrient uptake in marine organisms (Azam et al. 1983; Ouverney and Fuhrman 2000; Fenchel 2008). For marine metazoans, absorption of this component of the DOM pool has been shown to be energetically significant (Manahan et al. 1982, 1983). In eggs of *C. gigas*, amino acid transport is activated upon fertilization; in larval stages, the primary site of uptake from seawater is the ciliated velum (an organ involved in swimming and feeding; Manahan 1990). One key role for this transport process is starvation resistance when particulate food is absent, allowing for the maintenance of biomass (Moran and Manahan 2004).

In this study, we address the hypothesis that variance in transport physiology has a genetic component. Furthermore,

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we have identified a large number of amino acid transporter genes that are expressed during development of *C. gigas*. We conclude that this large number of genes makes it challenging to select "biomarkers" that are predictive of physiological rates and that direct in vivo measurements of transport are a better predictor of genetically determined variance in growth potential.

Material and Methods

Genetic Crosses

A series of full-factorial crosses was conducted using pedigreed lines of *Crassostrea gigas* that were genotyped using microsatellite and single nucleotide polymorphism markers (Hedgecock and Davis 2007; D. Hedgecock, personal communication). From these crosses, a total of 13 larval families were produced, and, based on growth rates, seven were selected to investigate genetically determined variation in growth rate and amino acid transport. A total of over 14 million larvae were cultured in large-scale experiments (ca. two million larvae per family) for the purpose of this study.

Larval Culturing

Gametes were strip-spawned from gravid males and females of different pedigreed lines. Eggs were fertilized and placed at a concentration of 10 eggs mL^{-1} in 200-L culture vessels containing 0.2-μm (pore-size) filtered seawater. Larval families were named according to the parental lines used for crosses. For example, larval family 3 × 9 (sire × dam) was a result of crossing an individual male and female from lines "3" and "9," respectively. The addition of "A" or "B" to a family name indicates a replicate cross of a male and female of the same pedigreed line. The 2,600 L of larval cultures were maintained in a temperature-controlled room that was continuously monitored using three temperature loggers (HOBO U12, Onset Computer, Bourne, MA) placed in different culture vessels. These readings over the experimental period of 16 d of culturing revealed that temperature was held constant at 24.5° ± 0.14°C (mean ± SEM). Larvae were competent to feed by 2 d of age, at which time they were fed the algae *Isochrysis galbana*. Algae were initially supplied at 30 cells μL^{-1}, and, as larvae grew, the ration was increased to 50 cells μL^{-1} according to established protocols (Breese and Malouf 1975). A complete replacement of the seawater in each 200-L culture vessel was performed every 2 d during experiments, using a custom-designed high-flow seawater filtration system located at the Wrigley Marine Science Center on Santa Catalina Island, California. Freshly filtered seawater was heated to the experimental culture temperature using titanium (inert) heat exchangers before being added to culture vessels.

Growth Rate

Growth rate was calculated from changes in larval shell length, defined as the distance from the anterior to posterior edge. Larvae were measured for at least six different ages over the 16-d developmental period studied. At each time point, images of 50 randomly selected individuals were captured using a digital camera (model DP72, Olympus) mounted to a compound microscope (model BX51, Olympus). Shell lengths were measured in calibrated photo-micrographic images using digital imaging software (DP2-BSW, Olympus). Shell length strongly predicts the protein content of larvae of *C. gigas*, including those from families that have genetically determined contrasts in growth rates (Pace et al. 2006). Over 2,300 individual shell length measurements were made during the course of these experiments. Growth rates ($\mu m\ d^{-1}$ ± SE of slope) were calculated for each larval family based on the slopes of linear regression models for changes in shell length with time.

Amino Acid Transport

Previous analyses of the Michaelis-Menten kinetics (affinity, K_t; maximum capacity, J_{max}) of neutral amino acid transport in larvae of *C. gigas* (Manahan 1989; Manahan et al. 1989) were used to design the current experiments. Two concentrations (10 and 100 μM) of the neutral amino acid glycine were selected to assess the effect of size and genotype on transport rates. A glycine concentration of 10 μM is near the K_t of amino acid transport in larvae of *C. gigas*; a concentration of 100 μM yields maximum transport rates (J_{max}). A total of 136 time-course transport assays (six individual time points per assay) were conducted according to the method described previously (Pace et al. 2006). For each assay, larvae were incubated in 10 mL of filtered seawater at 25°C with known amounts of radioactivity (range was 37–74 kBq ^{14}C-glycine, Perkin Elmer, Santa Clara, CA) with appropriate amounts of "cold carrier" to adjust to the desired substrate concentration (i.e., nonradioactive glycine added, Sigma-Aldrich, St. Louis, MO). For each transport assay, a well-mixed aliquot of seawater with known number of larvae was collected every 6 min and passed through an 8-μm (pore size) membrane filter (Nucleopore, Pleasanton, CA). Larvae were retained on the filter and rinsed with 10 mL of filtered seawater to remove the incubation medium containing excess radioisotope. The filter with larvae was transferred to a 7-mL scintillation vial containing 500 μL of tissue solubilizer (Solvable, Perkin Elmer, Billerica, MA); larval tissue was solubilized in the vial overnight. After the addition of liquid scintillation cocktail, the amount of radioactivity in each sample was measured using a quench-corrected liquid scintillation counter (Beckman Coulter, Brea, CA). The amount (moles) of glycine transported by larvae was calculated for each sample from the radioactivity per larva corrected for the specific activity of ^{14}C-glycine in seawater. Transport rates (pmole glycine $larva^{-1}$ h^{-1} ± SE of slope) were determined based on the slopes of linear regression models for changes in the accumulation of total glycine with time.

Amino Acid Transporter Genes

Genes encoding neutral amino acid transporters have been cloned in developmental stages of marine invertebrates; Meyer

and Manahan (2009) characterized three distinct amino acid transporter genes in embryos and larvae of the sea urchin, *Strongylocentrotus purpuratus*. Analysis of these genes revealed them to be members of the Amino Acid Transporter I (AATI) and *inebriated* (INE) subfamilies of the SoLute Carrier Family 6 (SLC6). The amino acid sequences deduced from full open reading frames of these three sea urchin amino acid transporter genes, as well as 11 additional sequences cloned from other echinoderms (Applebaum et al. 2013), were used as query sequences in National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990) searches of the *C. gigas* genome. This analysis resulted in the identification of 45 genes with similarity to SLC6 transporters. These *C. gigas* sequences were subsequently used in BLAST searches to query the *C. gigas* genome for any additional similar sequences (no additional sequences were found). These 45 genes were evaluated by the Conserved Domain Architecture Retrieval Tool (Geer et al. 2002) and reduced to 44 sequences that had structural organization consistent with the SLC6 family. Alignments (MacVector 10.6.0 software) of these 44 transporters revealed three duplicates, reducing the candidates to 41. BLAST search of the NCBI nonredundant protein databases was used to categorize these 41 genes into SLC6 subfamilies. Subfamily membership was assigned based on the top BLAST result for a gene functionally validated by physiological assay. A total of 23 amino acid transporter genes in the AATI and INE subfamilies were identified in the genome of *C. gigas* based on this analysis.

To verify that these 23 genomic DNA genes are expressed during development, a search of a published transcriptomic database for 34 embryonic and larval stages of *C. gigas* (table S14 in Zhang et al. 2012) was conducted. The presence or absence of each transcript was assessed during three periods of development: embryos (10 developmental stages: 0 to <9.5 h old), trochophore larvae (five developmental stages: 9.5 to <15.5 h old), and veliger larvae (19 developmental stages: 15.5 h old to 18 d old). Expression of a specific transcript was considered to be present in embryos or larvae if it was detected in at least one developmental stage within the developmental periods outlined above. Zhang et al. (2012) state that gene expression is detectable when reads per kilobase per million mapped reads is greater than one.

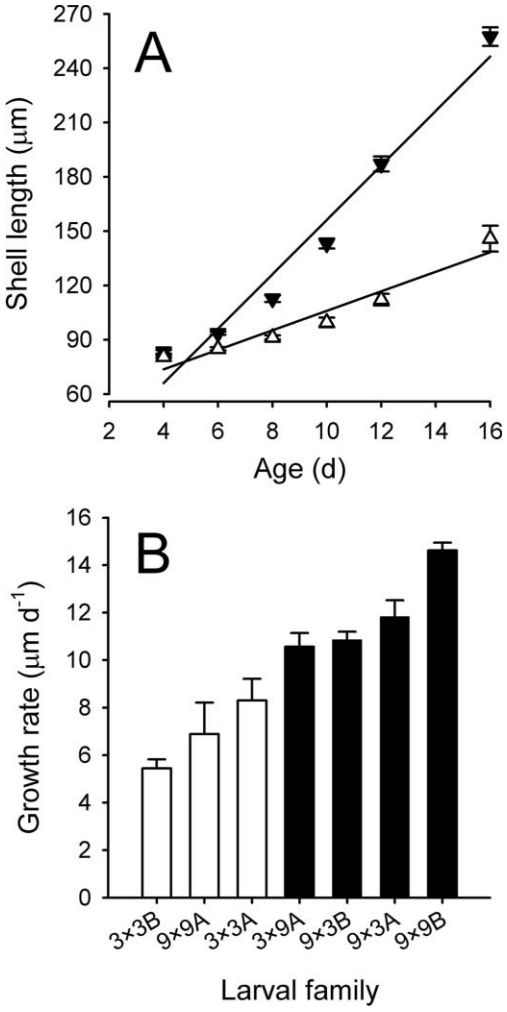

Figure 1. Growth of larvae of *Crassostrea gigas*. *A*, Larval shell length as a function of age for the slowest-growing (3 × 3B, open triangles) and fastest-growing (9 × 9B, filled triangles) families tested. The regression equations describing relationships between shell length and age are 3 × 3B, shell length = 5.4(age) + 51.5 (SE of slope = 0.38); 9 × 9B, shell length = 14.6(age) + 10.8 (SE of slope = 0.33). Error bars represent SEM; $N = 50$. Where not visible, error bars fell within the graphical representation of the data point. *B*, Calculated growth rate for the seven larval families. Each is designated by a male-by-female cross; "A" and "B" by each cross represent larval families originated from crosses of different adults within the same pedigreed line. Growth rate (slope ± SE of slope) for each of seven families is determined from the linear regression of increase in shell length with age.

Results

Growth Rate

Across all larval families generated from crosses of pedigreed lines of *Crassostrea gigas*, the maximum contrast in daily growth rate was a 2.7-fold difference between families 9 × 9B (14.6 ± 0.33 μm d^{-1}, slope ± SE) and 3 × 3B (5.4 ± 0.38 μm d^{-1}; fig. 1*A*). The ranking of growth rates for the seven families analyzed generally shows the pattern that hybrid families had the higher growth rates (fig. 1*B*: the single exception being family 9 × 9B). We do not have a definitive explanation for the growth contrast between the two sets of crosses of family 9 × 9 (A and B) generated from different parents. To eliminate the possibility of misidentified families, and to confirm pedigree, larvae from those two families were genotyped. While adults from the same pedigreed line represent closely related individuals, they are not genetically identical. It is likely that different adults from the same pedigreed lines may contain rare genetic characteristics that result in progeny with high variance and unexpected phenotypes. Nonetheless, the contrasting phenotypes are still genetically determined and are of interest for further physiological characterization. Having established genetically determined contrasts in growth rate, we next investigated the potential for variation in transport physiology associated with differences in growth among families.

Amino Acid Transport

Glycine transport rates at both concentrations tested (10 and 100 μM) showed a positive and linear relationship with increasing shell length (fig. 2). At 100 μM, maximum transport capacity (J_{max}) is attained (Manahan 1989; Manahan et al. 1989); that is, transport at 100 μM glycine is at saturation for a neutral amino acid. The equations describing ontogenetic changes in glycine transport rates are: $J_{10\ \mu M} = 0.05$(shell length) $- 2.60$ (ANOVA, $r^2 = 0.86$, $P < 0.001$, 68 assays) and $J_{max} = 0.24$(shell length) $- 14.84$ ($r^2 = 0.87$, $P < 0.001$, 68 assays). Transport rates at 10 μM glycine showed an eightfold increase, based on the linear regression of rates, from 1.3 pmole larva^{-1} h^{-1} at 2 d old to 10.3 pmole larva^{-1} h^{-1} at 16 d old (fig. 2: day 2 size $=$ 73.5 $\pm$ 0.45 μm; day 16 size $=$ 257.5 $\pm$ 5.14 μm). Over the same range of larval sizes, glycine transport rates at the higher concentration of 100 μM increased 16-fold from 3.0 to 48.2 pmole larva^{-1} h^{-1}. These results indicate that during development, transport rate at higher substrate concentration increases disproportionately to transport at a lower substrate concentration (16- vs. eightfold).

Genotype-Dependent Amino Acid Transport Rate

For the initial analysis, the differences in substrate-concentration-dependent transport rates were pooled independent of family (fig. 2). Further analysis as follows reveals that genotype changes transport rate. Primary data for glycine transport at 100 μM show that rates for family 3 $\times$ 9A were twofold higher than those for family 3 $\times$ 3A (fig. 3*A*, slope differences, $P < 0.001$). However, these two families of the same age (10 d old) had significant differences in size (3 $\times$ 9A $=$ 152.6 $\pm$ 1.66 μm; 3 $\times$ 3A $=$ 121.7 $\pm$ 3.42 μm). To correct for size-dependent changes in physiological rates, transport rates are expressed on a per unit shell length basis (fig. 3*B*, 3*C*). Size-specific transport rate at 100 μM was significantly higher in faster-growing genotypes (fig. 3*B*). This conclusion is based on an ANCOVA, in which slopes are parallel ($P = 0.35$), and importantly, the *Y*-axis intercepts are statistically different ($P < 0.001$), indicating a size-at-age-specific difference in glycine transport rate. This difference as mentioned above is genotype dependent. There is also no difference in rates (slopes, $P = 0.23$) for the same genotypes at 10 μM glycine. In contrast to larvae exposed to 100 μM, at 10 μM analysis of the age-corrected means reveals overlaps of the 95% confidence intervals of mean size-specific $J_{10\ \mu M}$ in faster- and slower-growing families (mean $\pm$ 95% CI $=$ 0.031 $\pm$ 0.0020 and 0.027 $\pm$ 0.0024 pmole μm^{-1} h^{-1}, respectively). This analysis reveals that the size-specific, genotype-dependent higher transport rate is only evident at the higher substrate concentration. This suggests a genotype-dependent change in maximum transport capacity (J_{max}).

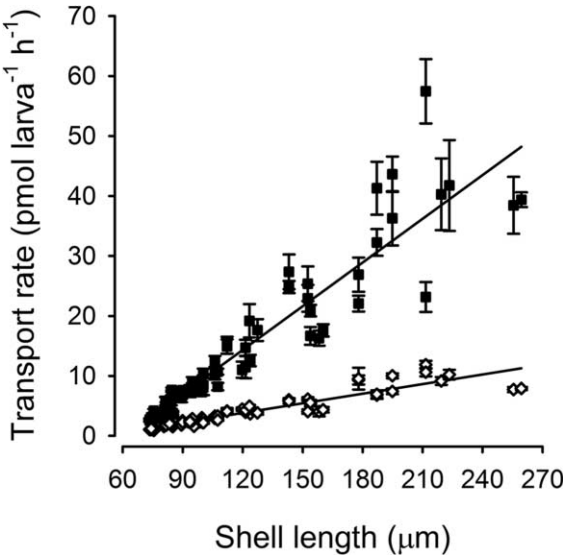

Figure 2. Glycine transport rates of larvae of *Crassostrea gigas*. Rates measured at 10 μM glycine ($J_{10\ \mu M}$, open symbols) and 100 μM glycine (J_{max}, filled symbols) as a function of shell length. Each data point represents rate of glycine transport ($\pm$SE of slope) calculated from the slope of a six-point time-course transport assay; where not visible, error bars fell within the graphical representation of the data point. The regression equations describing relationships between transport rate and shell length are $J_{10\ \mu M} = 0.05$(shell length) $- 2.60$ (SE of slope $=$ 0.003) and $J_{max} = 0.24$(shell length) $- 14.84$ (SE of slope $=$ 0.012).

Amino Acid Transporter Genes

Genotype-dependent variations in amino acid transport capacity (fig. 3*B*) may be associated with changes in expression of transporter genes. Surprisingly, we have identified 23 distinct transcripts from the genome as putative amino acid transporter genes in developmental stages of *C. gigas* (table 1). Our analysis of a published transcriptomic database reveals that 15 of these transporter genes are expressed in embryo stages, with four additional genes expressed in trochophore stages, and the full complement of 23 genes being expressed in the veliger stages. Clearly, the molecular biological bases of transport physiology and its regulation are highly complex in developmental stages of *C. gigas* (table 1).

Discussion

The present work investigated the genotype-dependent variation in fundamental physiological processes during development—specifically, growth and nutrient intake. Taking advantage of genetically determined phenotypic differences among larval families, we found that transport capacity increased more rapidly with size in faster-growing families (fig. 3*B*). This observation supports the hypothesis that variation in transport physiology during larval growth has a genetic basis. We also discuss the application of molecular biological versus physiological "biomarkers" for growth potential.

Ontogenetic Changes in Amino Acid Transport Physiology

A linear and positive relationship between transport capacity and larval growth is a common phenomenon in developing marine invertebrates (fig. 2; Manahan et al. 1989; Manahan

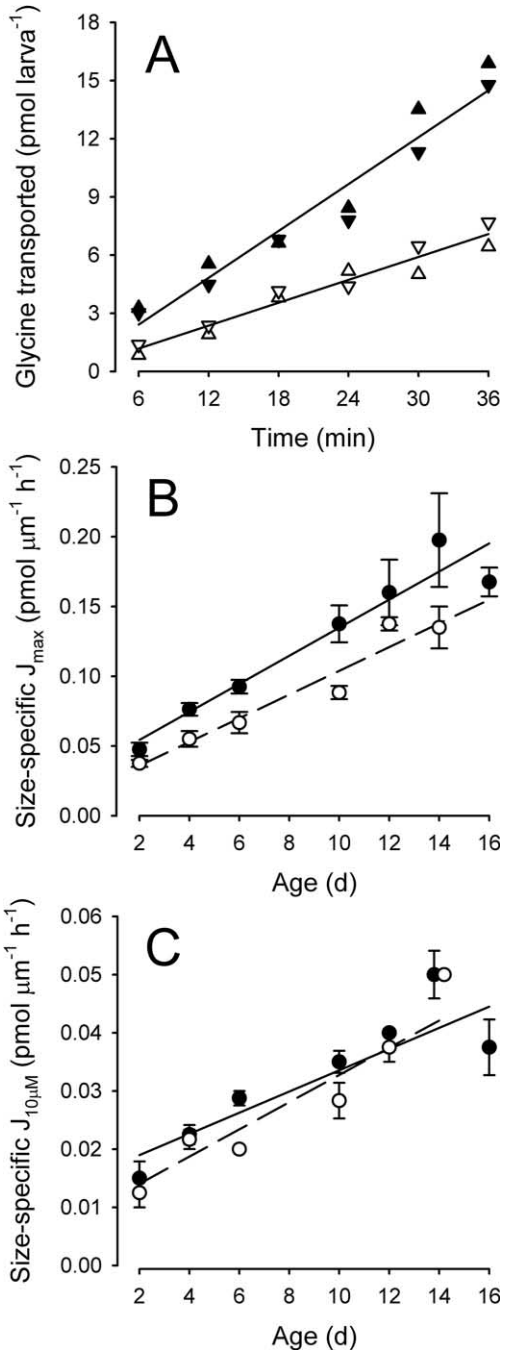

Figure 3. Glycine transport rates of faster- and slower-growing larval families of *Crassostrea gigas*. *A*, Time course of glycine transport from 100 μM glycine by 10-d-old larvae from two families, 3 × 3A (open triangles) and 3 × 9A (filled triangles). Transport rates (± SE of slope) were determined in replicate assays (triangles and inverted triangles) within each family. Rates were divided by shell length to determine size-specific transport rates given in *B* for 100 μM and in *C* for 10 μM glycine. Each data point represents mean size-specific transport rate (± SEM) for faster-growing (filled circles) and slower-growing (open circles) families. Faster-growing families showed higher size-specific maximum transport capacity (J_{max}) than the slower-growing families (*B*: by ANCOVA parallel slopes: $P = 0.35$, *y*-intercept: $P < 0.001$).

1990; Pace et al. 2006). In previous studies (Manahan et al. 1989; Pace et al. 2006), we showed that there was a linear relationship between transport rate and size for neutral amino acid transport. We have significantly extended that prior work with the findings that (i) there is a different scaling relationship for transport at lower concentration (10 μM) compared to higher concentration (100 μM; fig. 2); (ii) there is a genetically determined, size-specific transport capacity (fig. 3*B*); and (iii) there is a relationship between growth and amino acid transport that may serve as an early predictive index of growth rate (fig. 4). The developmental increase in transport capacity is likely to be a result of the increase in transport surface area of the velum. Additionally, the size-specific increase in maximum transport capacity in faster-growing genotypes is possibly linked to increased number of amino acid transporter proteins in membrane. Putative genes encoding for these proteins are now starting to be characterized (table 1).

Genomic Analysis of Amino Acid Transporters

There is considerable interest in the identification of genes that regulate physiological processes and how animals adapt to the environment (Whitehead et al. 2011; Somero 2012). As the technical and economic barriers to quantifying steady state expression of individual transcripts (quantitative polymerase chain reaction) or the whole transcriptomic profiling (mRNA sequencing) of organisms have been overcome, efforts to use gene expression as an indirect measure of physiological status have intensified (Chapman et al. 2011; Konopka and Wilkins 2012). There has been a massive expansion in genomic information, even for "nonmodel" species of interest to comparative biologists (Cossins and Somero 2007). A central theme of this "-omic" approach has been to identify genes that may be functionally linked to specific physiological processes, and whose expression could reflect physiological and biochemical rates. We now discuss an integrative approach to have a predictive basis to link genetically determined variance in organismal-level growth, through organ-level nutrient transport physiology, to genes.

We searched the genome of *C. gigas* (Zhang et al. 2012) for putative members of the amino acid transport subfamilies Amino Acid Transporter I (AATI) and *inebriated* (INE), within the SoLute Carrier 6 family (SLC6). Proteins encoded by genes in these subfamilies have been shown to be localized in the body-wall surface ectoderm of developing sea urchins (Meyer and Manahan 2009). Based on gene constructs and heterologous expression, these sea urchin genes have been demonstrated to transport neutral amino acids, confirming the identification of genes involved in the direct uptake of dissolved organic material from seawater. In the current study, our systematic search identified 23 genes that had sequence and domain architecture consistent with membership in the AATI or INE subfamilies of the SLC6. Although the majority of these gene predictions have not been validated through cloning of the expressed transcript, it is important to note that each of the 23 genes is detected in a transcriptomic analysis of developmental stages of *C. gigas*. This additional bioinfor-

Table 1: Expression during development of the Pacific oyster, *Crassostrea gigas*, of 23 putative SoLute Carrier Family 6 genes of the Amino Acid Transporter I (AAT1) and *inebriated* (INE) subfamilies

C. gigas gene accession number	SwissProt BLAST result	Embryo	Trochophore	Veliger
EKC17913.1	Na^+-dependent proline transporter, *Rattus norvegicus*	+	+	+
EKC29047.1	Na^+- and Cl^--dependent glycine transporter 1, *Xenopus laevis*	+	+	+
EKC17288.1	Na^+- and Cl^--dependent glycine transporter 2, *Homo sapiens*	+	+	+
EKC24822.1	Na^+- and Cl^--dependent glycine transporter 2, *Mus musculus*	+	+	+
EKC22426.1	Na^+- and Cl^--dependent glycine transporter 2, *H. sapiens*	+	+	+
EKC35081.1	Na^+- and Cl^--dependent glycine transporter 2, *H. sapiens*	+	+	+
EKC36022.1	Na^+- and Cl^--dependent glycine transporter 2, *X. laevis*	+	+	+
EKC36021.1	Na^+- and Cl^--dependent glycine transporter 2, *H. sapiens*	+	+	+
EKC33781.1	Na^+- and Cl^--dependent glycine transporter 1, *X. laevis*	ND	ND	+
EKC31399.1	Na^+- and Cl^--dependent glycine transporter 1, *X. laevis*	+	+	+
EKC40069.1	Na^+- and Cl^--dependent glycine transporter 2, *H. sapiens*	ND	+	+
EKC24094.1	Na^+- and Cl^--dependent glycine transporter 2, *H. sapiens*	+	+	+
EKC30006.1	Na^+- and Cl^--dependent glycine transporter 2, *H. sapiens*	+	+	+
EKC25312.1	Na^+- and Cl^--dependent glycine transporter 2, *X. laevis*	ND	ND	+
EKC21522.1	Na^+- and Cl^--dependent glycine transporter 2, *H. sapiens*	ND	ND	+
EKC33430.1	Na^+- and Cl^--dependent *inebriated*, *Drosophila melanogaster*	+	+	+
EKC37562.1	Na^+- and Cl^--dependent glycine transporter 2, *M. musculus*	ND	+	+
EKC37563.1	Na^+- and Cl^--dependent glycine transporter 2, *H. sapiens*	ND	+	+
EKC18061.1	Na^+- and Cl^--dependent glycine transporter 2, *M. musculus*	ND	ND	+
BAF02549.1	Na^+- and Cl^--dependent glycine transporter 2, *M. musculus*	+	+	+
EKC35348.1	Na^+- and Cl^--dependent glycine transporter 2, *H. sapiens*	+	+	+
EKC40294.1	Na^+- and Cl^--dependent glycine transporter 2, *M. musculus*	ND	+	+
EKC22732.1	Na^+- and Cl^--dependent glycine transporter 2, *H. sapiens*	+	+	+

Note. Most similar SwissProt BLAST results (Zhang et al. 2012) for each *C. gigas* gene are given. A plus sign indicates when a specific gene is expressed during each of three developmental periods. ND indicates gene expression not detected. Gene expression data were obtained from a published transcriptomic data set (Zhang et al. 2012, table S14).

matic analysis confirms that these genes are expressed during development and could be investigated for their role in physiological transport systems.

The size of this amino acid transport gene complement in *C. gigas* complicates any attempt to use steady state gene expression levels as an indicator of physiological status (i.e., a proxy for transport physiology). The combinatorial multiplicity of the 23 genes identified necessitates specific characterization of the role of each gene in the physiology of interest. Such characterization needs to encompass studies of histospecific spatial expression and the functional analysis of the kinetics and substrate preference of each transporter protein (cf. sea urchins; Meyer and Manahan 2009). The situation is even more complex when one considers the possibility of additional genes from other amino acid transporter families. For instance, there are several other gene families that have been well characterized in mammals, including sodium-dependent cotransporters for neutral amino acids. These include monomeric transporters within families SLC1 and SLC38, as well as the heteromeric transporter families SLC3 and SLC7 (Bröer 2008; He et al. 2009; Stevens 2010). The numbers of putative AATI and INE genes identified in *C. gigas* are much greater than those known to exist in the genomes of mammals. For instance, in humans the AATI subfamily includes only four genes (Bröer and Gether 2012). It appears that expansion of gene families is a common occurrence in the *C. gigas* genome. That genome contains 88 heat shock protein 70 genes and 133 cytochrome P450 genes; in contrast, sea urchins have 39 heat shock protein 70 genes, and humans have ~17 heat shock protein 70 genes and ~65 cytochrome P450 genes (Zhang et al. 2012). These recent findings highlight the expansion and multiplicity of potential regulators of physiological processes at the molecular biological level in *C. gigas*. This high level of genetic complexity identified in this species for which there is a recently sequenced genome suggests that caution is warranted for other "nonmodel" organisms of interest to comparative biologists. Lacking genomic information, the extent and complexity of gene families are unknown, thus making difficult the selection of genes predictive of organismal responses to environment.

Amino Acid Transport Physiology as a Predictor for Growth Potential

Our analysis shows that size-specific amino acid transport capacity has a genetic basis (fig. 3*B*). The parallel nature of the size-specific slopes shows that the greater transport capacity in faster-growing larvae is set very early in development. Interestingly, this capacity for acquisition of dissolved nutrient is similar in profile to that of particulate feeding (Pace et al. 2006).

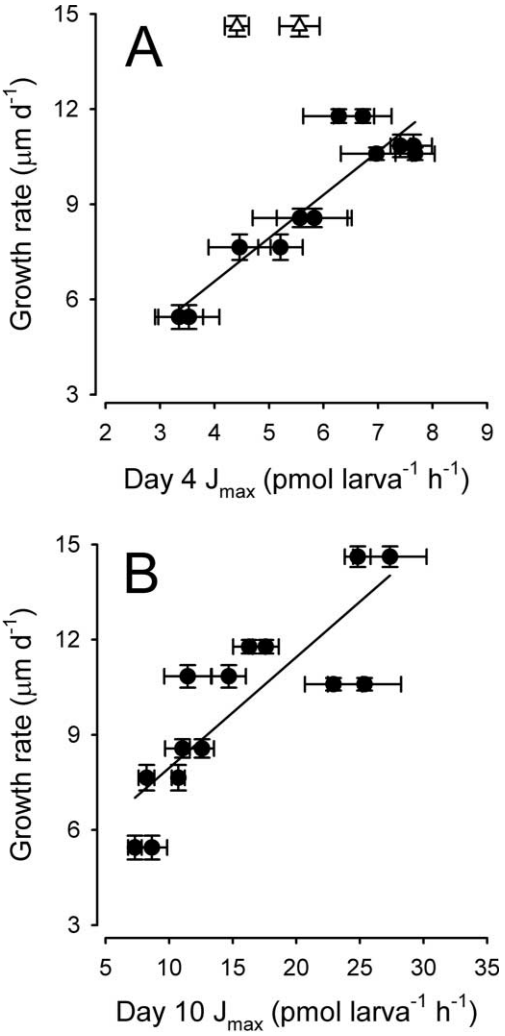

Figure 4. Maximum transport capacity (J_{max}) as a predictor of growth rate in larvae of *Crassostrea gigas*. Relationships between growth rate and J_{max} for (*A*) 4-d-old larvae and (*B*) 10-d-old larvae. Data points represent J_{max} (±SE of slope) and the corresponding growth rate (±SE of slope) for each larval family. In *A*, larval family 9 × 9B (open triangles) is excluded from the linear regression model used to fit the line shown (see "Discussion" for rationale). The regression equations describing relationships between growth rate and J_{max}: *A*, growth rate = $1.4J_{max}$ + 1.1 (SE of slope = 0.19); *B*, growth rate = $0.3J_{max}$ + 4.5 (SE of slope = 0.07).

It is noteworthy that there is a genetic component for both particulate feeding and dissolved nutrient uptake, which results in a size-specific difference in the ability to acquire nutrients during development (i.e., parallel slopes with respect to size). As was elegantly highlighted by Schmidt-Nielsen (1984) and Weiss et al. (1998), our understanding of the scaling principles of energy input (e.g., feeding) are less well developed than those of energy output (e.g., respiration). Studies of the physiological capacities of developmental forms that have high rates of growth, with corresponding requirements for high rates of energy input, are uniquely posed to help address this coscaling issue. In general, the prediction of variation in subsequent growth rate from very early developmental stages (e.g., embryos of similar size) remains challenging. For developmental stages of *C. gigas*, it is not possible to resolve genetically determined growth differences within the first few days of development based on morphological measurements (all 2-d-old veliger larvae are about the same size). Here we propose the possibility of developing a predictive physiological index for differential growth potential. This analysis is based on combining the genetically determined contrasting growth phenotypes (fig. 1*B*) with size-specific differences in transport rates (fig. 3*B*). Maximum transport capacity in 4-d-old larvae is a good predictor of subsequent growth out to 16 d for six of seven families tested (fig. 4*A*: based on r^2, 83% of growth rate variation explained). The fastest-growing family, 9 × 9B, is excluded from this analysis (the regression shown is for six families). There is no a priori reason to assume that the model proposed should predict all genetically determined growth variance at such an early stage of development. However, even at such an early stage of development, the model is quite robust and can predict growth potential for six out of seven families. Once a later larval stage is reached (10 d old), prediction improves for all seven families (fig. 4*B*: $r^2 = 0.70$). While there is a predictive relationship between maximum transport capacity and growth, we are not suggesting that differential growth is solely driven by the acquisition of dissolved organic material from seawater.

Defining genetically determined variance is of great interest for hypothesis testing of "nature" versus "nurture" scenarios. Specifically, in developmental physiology understanding variation in growth has many applications. Yet dissecting nature from nurture and developing appropriate biomarkers remain elusive. Even in highly studied fields such as cancer, the prediction of tumor growth potential based on biological markers remains challenging (Curtis et al. 2012). For other important applications such as food production, predicting growth potential from seeds to agricultural production is the basis of a major agribusiness industry (hybrid corn). Given the global economic importance of oyster culture (Food and Agricultural Organization 2012), there is obviously a parallel consideration of viewing larvae as "seeds." Our study illustrates that the combination of quantitative genetics, developmental biology, and physiology will increase the possibility of being able to predict differential growth, starting with the earliest stages of development. It is unlikely, however, that such predictive power will emerge solely from the analysis of gene expression, given the diversity of gene families in *C. gigas* involved in amino acid transport and the numbers of genes in excess of dozens. Studies that combine in vivo analyses of physiological traits with genomics and genetics are likely to provide a promising way forward. The complexity of gene complements that influence organismal physiology could be reduced by applying quantitative trait loci analysis, in which phenotypic traits are mapped to specific regions of the genome (Doerge

2002; Plough and Hedgecock 2011; Applebaum et al. 2014). For this and other applications, the further development of model organisms with well-developed genomic, genetic, and physiological resources will greatly assist the ability to address complex interactions of exogenous environmental and endogenous biological factors that regulate development and growth.

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