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SYMPOSIUM

Separating the Nature and Nurture of the Allocation of Energy in Response to Global Change

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Synopsis Understanding and predicting biological stability and change in the face of rapid anthropogenic modifications of ecosystems and geosystems are grand challenges facing environmental and life scientists. Physiologically, organisms withstand environmental stress through changes in biochemical regulation that maintain homeostasis, which necessarily demands tradeoffs in the use of metabolic energy. Evolutionarily, in response to environmentally forced energetic tradeoffs, populations adapt based on standing genetic variation in the ability of individual organisms to reallocate metabolic energy. Combined study of physiology and genetics, separating "Nature and Nurture," is, thus, the key to understanding the potential for evolutionary adaptation to future global change. To understand biological responses to global change, we need experimentally tractable model species that have the well-developed physiological, genetic, and genomic resources necessary for partitioning variance in the allocation of metabolic energy into its causal components. Model species allow for discovery and for experimental manipulation of relevant phenotypic contrasts and enable a systems-biology approach that integrates multiple levels of analyses to map genotypic-to-phenotypic variation. Here, we illustrate how combined physiological and genetic studies that focus on energy metabolism in developmental stages of a model marine organism contribute to an understanding of the potential to adapt to environmental change. This integrative research program provides insights that can be readily incorporated into individual-based ecological models of population persistence under global change.

Introduction

Intense, human-induced changes to the earth occurring on a geologically and evolutionarily rapid time scale (~300 years) prompted Crutzen (2002) to suggest that we live in a new geological epoch—the Anthropocene. As a consequence of ever-increasing impacts of human activity on the biosphere, understanding how biology will be affected on a changing planet has become a leading area of emphasis for researchers in the environmental life sciences (Parmesan and Yohe 2003; Hoffmann and Sgrò 2011; Somero 2012; Intergovernmental Panel on Climate Change 2014). A primary objective is to predict how projected, anthropogenic global environmental changes will shape the biology of individuals, populations, species, and ecosystems. Are individual organisms capable of maintaining biological stability and coping with changing environmental conditions? Or, alternatively, can adaptation through evolutionary selection match the pace of global environmental change? This "Nature versus Nurture" issue (Galton 1895) is fundamental in evolutionary biology. Here, we advocate for a Darwinian approach that allows for a quantitative analysis of biological variance in response to environmental change. In our view, this can best be achieved by combining studies at genetic and physiological levels (i.e., separating the "Nature and Nurture" of biological responses).

Stability and change: evolution based on standing variation

Evolution by natural selection occurs because organisms have excess reproductive capacity and

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individuals of the same species vary extensively. A major goal of research on biological responses to global change is to predict long-term responses of species, and their populations. However, the principles of Darwinian biology often are challenging to apply in studies of this kind because of the difficulties of isolating variation in biological responses that are associated with genotype, environment, and the interaction of these causal factors.

In experiments with groups of conspecific, wild-type animals, perturbations of a wide range of biotic (e.g., food) or abiotic (e.g., temperature) variables may elicit a significant, mean biological response. However, variability associated with these means often is large and has a genetic component. Pooling wild-type animals may mask genotypic variance in biological response to a hypothetical future environment (Fig. 1, left panel), such that there may be little, or no, statistical change in mean response (measured as a fitness-related trait, such as growth or survival). However, if the responses of individual genotypes are known, a very different pattern may emerge (Fig. 1, right panel). One genotype, for instance, may have increased fitness (*thrives*), whereas another may have decreased fitness (*suffers*) and a third genotype may show no change (*unaffected*). Thus, by partitioning the biological variance of wild-type populations, new insights can emerge regarding biological potential to respond to environmental change. In quantitative genetic terms, the crossing pattern (Fig. 1, right panel) in the responses of genotypes, going from present to future conditions, is a genotype-by-environment interaction (Lynch and Walsh 1998).

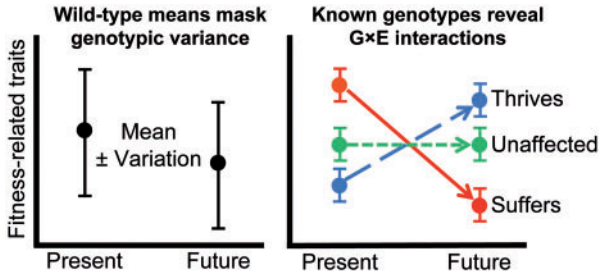

Fig. 1 Biological variation in the response of fitness-related traits to environmental change. Hypothetical representation of possible responses of a wild-type animal population to *Present* and *Future* environmental conditions. Using wild-type animal populations (left panel; *wild-type means mask genotypic variance*), there is a slight negative mean response that is not statistically significant (note error bars). Using known genotypes (right panel; *known genotypes reveal G × E interactions*), a range of responses reveals a genotype-by-environment interaction and how each genotype *thrives*, is *unaffected*, or *suffers* in a changing environment.

Metabolic basis of maintaining homeostasis in response to environmental change

The principles of "*How Animals Work*" (Schmidt-Nielsen 1972) to achieve changes in body size, form, function, and energy supply under changing environmental conditions are central to evolutionary physiology. In physiological terms, the basis of adaptation is an organism's ability to maintain homeostasis by allocating resources without impeding other biological functions (Schmidt-Nielsen 1972; Hochachka and Somero 2002). Clearly, there is likely to be a metabolic basis to fitness-related traits. Compounded on this are environmental perturbations that alter physiological and biochemical *milieu*. Such responses often have associated metabolic changes in total demand or allocation of energy. Shifts and tradeoffs in allocation of energy in response to factors associated with global environmental change are known to directly affect the availability of energy for physiological processes (Hawkins et al. 1987; Langenbuch and Pörtner 2003; Cherkasov et al. 2006; Deigweiher et al. 2010; Guderley and Pörtner 2010; Sokolova et al. 2012).

The differential regulation and allocation of metabolic energy to fuel cellular and systemic work are also a central theme in biology (Kleiber 1961; West et al. 1999; Darveau et al. 2002). High-energy adenosine-triphosphate (ATP) molecules are the common energy currency in cellular metabolism. An organism's ATP pool must be partitioned among all of its energy-consuming physiological and biochemical processes (Hulbert and Else 2000; Rombough 2006; Nelson et al. 2008). Biological responses to shifts in environmental conditions frequently cause changes in metabolic demand and ultimately tradeoffs in allocation of ATP. For instance, an environmental challenge that increases the demand for ATP for physiological maintenance may limit the allocation of ATP to growth, reproduction, or locomotion (Guderley and Pörtner 2010). Although metabolic cost often is invoked as an explanatory basis for organismal-level changes, the energetic bases of such tradeoffs are difficult to measure.

Furthermore, as illustrated (Fig. 1), genetic components are likely to have a major role in allocation of energy and in tradeoffs in response to environmental change. To address this issue, species with well-developed genetic, genomic, and physiological resources are needed to allow for the partitioning of phenotypic variance into its causal components. Such partitioning will facilitate a quantitative

understanding of mechanisms, thereby providing better predictions of the potential for adaptation.

Bridging organismal and cellular biology

Experiments conducted using isolated cells or tissues have provided remarkable insights into cellular and subcellular functions (Alberts et al. 2002). In studies of organismal biology, however, such approaches often are viewed as being too ''reductionist'' as they cannot provide an integrative biological view of the functioning of complex animal systems. The field of developmental biology offers a possible bridge between these two points of view. Many animals have complex life histories that involve larval stages of development. Larval forms are ''whole organisms'' with highly differentiated tissues, organs, and behaviors, while offering the experimental tractability so commonly sought in cell biology (e.g., ability to culture large numbers under controlled laboratory conditions; small size for ease of manipulation; and ability to perfuse with chemical treatments). Research using developmental stages of animals as model experimental subjects has facilitated a whole-organism approach for studies of the capacity to respond to environmental stress. Developmental stages of many species are now in common use for a wide variety of environmental studies related to impacts of biological, chemical, and physical parameters (Gilbert and Epel 2009).

Physiological ecology of development

The study of developmental biology has provided remarkable understanding of the endogenous mechanisms driving cellular differentiation and morphogenesis (Davidson 2001). However, the role of environment in shaping phenotype, although recognized by early embryologists (Hertwig 1894; Johannsen 1909), has only recently emerged as the field of ''Ecological Developmental Biology'' (Gilbert and Epel 2009). The biological response of organisms to global environmental change is currently the focus of intense research. Reviews and meta-analyses, integrating the results of large numbers of studies, indicate that developmental stages are sensitive to stress associated with global environmental change (Kroeker et al. 2010, 2013; Dupont et al. 2010; Kingsolver et al. 2011). Marine animals offer particular advantages in the field of ecological developmental biology, as the majority of species have a life-history strategy that includes a dispersive, planktonic larval stage (Thorson 1950; Crisp 1974; Cowen et al. 2006).

A marine model organism

Model organisms such as fruit flies and nematode worms have allowed for major advances to be made in the study of genotype–phenotype relationships. Most model organisms are terrestrial. Less progress has been made in the study of marine animals, owing historically to the absence of any capability for experimental genetics, particularly with larger marine metazoans. This situation has changed dramatically over the past 40 years, with the exponential growth of aquaculture and attendant interest in domestication and breeding (Hedgecock 2012). Enabling dissection of genotype-dependent variance in response within the context of the ocean would allow for a quantitative prediction of the resilience of populations of marine animals to global environmental change (Fig. 1). In selecting a marine animal species for study, we considered several important criteria:

(1) Life-history features typical of large, marine metazoan animals. Many species are highly fecund (releasing millions of eggs per spawn) and experience high mortality of early life-history stages.

(2) A species that is tractable for large-scale, early life-history culture under controlled laboratory conditions. A large amount of biological material is needed to integrate classical biochemical and physiological measurements with organismal and ''-omic'' analyses.

(3) The ability for complete life-cycle propagation. This is required for production of pedigreed lines and to enable genetic analyses.

(4) A large number of pedigreed lines. The availability of pedigreed lines across multiple generations allows for repeatable experimental crosses and the reproducibility of studies on selected phenotypes (cf. biomedical models, such as mice; agricultural species, such as corn). A large number of lines increases the opportunity for maximizing phenotypic contrasts in experimental crosses.

(5) A sequenced genome. This facilitates integration of genetic maps with genome sequence for discovery of candidate genes and associated regulatory features.

With these criteria in mind, we focused our efforts on the Pacific oyster *Crassostrea gigas*. This species now has well-developed genetic, genomic, and physiological resources. The oyster is one of the most widely cultured marine species in the world

(Food and Agricultural Organization 2012). Our productive partnerships with mature aquacultural enterprises over the past two decades have established closed populations, originated breeding programs, and maintained pedigreed lines.

Separating nature and nurture: the importance of pedigreed lines

Distinction between an organism's phenotype and its genotype "remains basic for clear thinking about genetic and evolutionary problems" (Dobzhansky 1970). Since phenotype (P) is independently determined by genotype (G), environment (E), and their interaction ($G \times E$ or simply, I), as summarized in the linear model, $P = G + E + I$, then it follows that, in a population of organisms, phenotypic variance, V_P, is the sum of the variances of its components, $V_P = V_G + V_E + V_I$. Thus, a model species for research on organismal responses to global environmental change must be amenable to the methods of classical, quantitative genetics for partitioning variance in response into its genetically determined component (nature), its environmentally determined component (nurture), and their interaction. This partitioning of phenotypic variance is achieved primarily through measuring the resemblance of relatives in pedigreed natural populations (e.g., comparisons of identical and fraternal twins in human populations, which are not possible for natural populations of marine species) or in experimental populations created by controlled crosses. The latter approach requires large experimental crosses, producing tens to hundreds of families, which has been achieved with only a few marine species (e.g., Hedgecock et al. 1995; Langdon et al. 2003; Hedgecock and Davis 2007; Sunday et al. 2011; Gjedrem 2012).

Experimental crosses of wild-type animals have been undertaken to attempt to define the heritable variation in response to environmental change (Sunday et al. 2011; Foo et al. 2012; Parker et al. 2012; Kelly et al. 2013; Munday et al. 2013; Pespeni et al. 2013). In such experiments, a very large number of parents is required to adequately sample standing genetic variation. Even if this challenging criterion is fulfilled, wild-caught parents are unique genotypes, cannot generally be re-tested, and do not provide reproducible phenotypic contrasts for mechanistic analyses (see the "A systems-biology approach: integrating genetics, genomics, and physiology" section). To attain the latter requires either artificial selection of contrasting lines based on additive genetic variation, or crossbreeding of pedigreed inbred lines, to capture non-additive genetic components of phenotypic variance.

We initiated a systematic breeding program that provides access to numerous pedigreed inbred lines derived from natural populations (Hedgecock et al. 1995; Hedgecock and Davis 2007). Inbred lines have been used extensively to understand biological mechanisms in the fields of medicine and agriculture but are often regarded as too "abnormal" for studies of natural populations (see Pace et al. [2006], however, for evidence to the contrary). Our focus is on F_1 hybrids produced by crossing inbred lines. The resulting hybrids are essentially the equivalent of naturally occurring genotypes, because random mating among inbred lines restores Hardy–Weinberg equilibrium proportions of genotypes (Lynch and Walsh 1998). Moreover, while selection among inbred lines could, in principle, reduce or distort natural diversity, gross loss of inbred lines has not been obvious in our >25 years of breeding Pacific oysters. The great advantage of having inbred lines is that they make possible factorial (diallel) crosses, in which male and female parents from each line are crossed in all possible combinations (Fig. 2) to produce a large set of F_1 hybrids. Although, in nature, these genotypes would be represented by single individuals, in our experiments they are represented by large numbers of genetically similar full-siblings, allowing for physiological analyses of developmental stages. Moreover, the same diallel cross can be repeated at different times, in different years, or in different generations (Pace et al. 2006; Hedgecock and Davis 2007), highlighting a key advantage of having pedigreed lines. For example, larval families produced by diallel crosses typically show contrasting "growth phenotypes" (Fig. 2) that can be reproduced across successive generations. F_1 hybrid populations are thus a scalable and repeatable genetic resource for revealing standing genetic variation in response to environmental change.

Allocation of metabolic energy

As discussed above (under the "Metabolic basis of maintaining homeostasis in response to environmental change" section), genetically determined or environmentally induced phenotypic contrasts likely have a metabolic basis. Understanding the mechanistic basis of the underlying metabolic response requires building energy budgets for a whole organism that define allocation of energy (ATP) at several levels: (1) quantification of total use of energy (metabolic rate measured by respiration for aerobic organisms); (2) the biochemical bases for changes in utilization

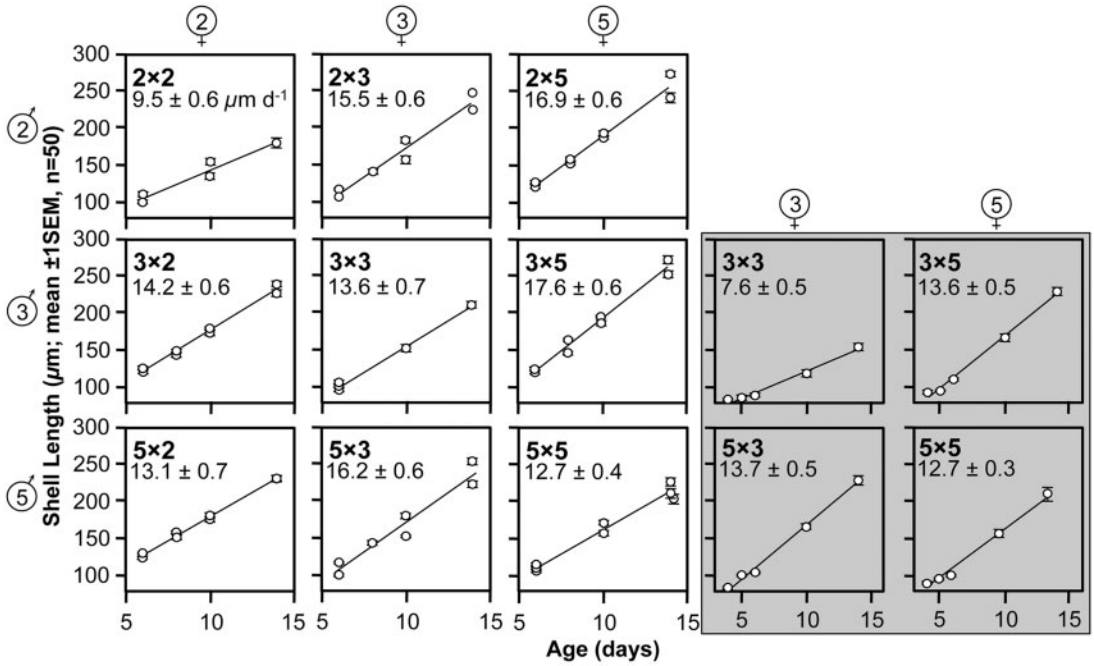

Fig. 2 Contrasting growth phenotypes in larvae produced by factorial crosses of pedigreed lines of the Pacific oyster *Crassostrea gigas*. Family names (e.g., top left panel, 2 × 2, sire × dam) and growth rates are given in the upper left of each panel (details in Pace et al. 2006). The graphs shown on the white background represent nine larval families from a single factorial cross. The four families within the gray rectangle represent a replicate cross of lines 3 and 5, produced from a subsequent generation of the same parental lines. Contrasts in growth phenotypes are also evident across parental generations.

of ATP; and (3) determination of the cost per unit activity of major ATP-consuming processes.

Few whole-organism studies apportion the ATP pool to specific biochemical processes. Although allocations of energy are tissue-specific and species-specific, there is general consensus that the dominant ATP-consuming processes in mammals are protein synthesis and ion transport (Siems et al. 1992; Buttgereit and Brand 1995; Rolfe and Brown 1997). Likewise, in sea urchin embryos, 40% of ATP is allocated to protein synthesis and a further 19% to sodium-pump activity (Na^+, K^+-ATPase), whereas in growing larvae, strikingly, up to 75% of available ATP is consumed by the process of protein synthesis alone (Fig. 3) (Leong and Manahan 1997; Pace and Manahan 2006). This high demand for energy to support growth is consistent with previous studies showing that larval developmental stages have higher specific growth rates than at any other life-history stage (Wieser 1994; Rombough 2006). These findings highlight distinct aspects of developmental physiology.

Genetically determined bases of physiological resilience

Our work has shown that genetically determined contrasting phenotypes of survival, growth, and their physiological bases can be repeatedly demonstrated by experimentally crossbreeding pedigreed lines of the Pacific oyster (Pace et al. 2006; Hedgecock et al. 2007; Curole et al. 2010; Meyer and Manahan 2010; Plough and Hedgecock 2011). We are presently extending this approach to define the physiological potential of organisms to allocate energy differentially in response to global change. Once energy budgets have been constructed for an organism (Fig. 3), a further step can be taken to investigate if there is a genotype-dependent shift in metabolic allocation of the major ATP-consuming processes (Fig. 4). We wish to know, for example, whether a genotype that *thrives* when the environment changes in some future scenario (Fig. 4; Genotype 1) has a different allocation of ATP than a genotype that cannot adapt (Fig. 4; Genotype 3 *suffers*), or that is *unaffected* by change (Genotype 2). We have preliminary data to show that such genetically determined variation in how an organism allocates energy in a changing environment does exist, even between paternal half-sib families. Such metabolic variation may be the basis of biological resilience (*thrives* and/or *unaffected*). Of particular note, here, is that these major changes in allocation of ATP and in potential for resilience would not be detected by simple measurements of total metabolic

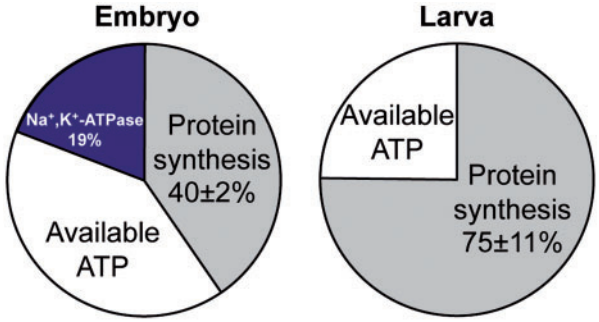

Fig. 3 Allocation of metabolic energy to protein synthesis and ion transport accounts for the majority of utilization of ATP in developmental stages of the sea urchin, *Lytechinus pictus*. Total available ATP, calculated from respiration rate, is represented by the area of the pie-chart for each developmental stage (not scaled across diagrams for increases in respiration between an *Embryo* and an exogenously feeding, growing *Larva*). Determination of allocation of ATP is based on *in vivo* measurements of protein synthesis rates and physiological flux of ion transport (Na^+, K^+-ATPase). For the *Larva* in this diagram only protein synthesis was measured, not the physiology of the sodium pump (details in Pace and Manahan 2006).

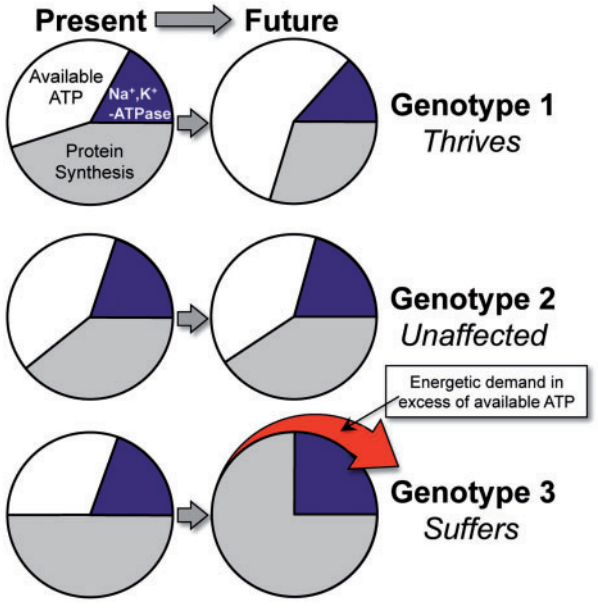

Fig. 4 Genotype-dependent changes in allocation of ATP in response to environmental change for individuals of three different genotypes. Conceptual diagrams in the left column illustrate metabolic allocation under *Present* conditions; those in the right column show the same genotype under a *Future* condition. Under a global-change scenario, genotype-specific responses of ATP allocation could result in an individual that *thrives* (*Genotype 1*: decreased demand for some energy-consuming processes, thereby increasing available ATP), an individual that is *unaffected* (*Genotype 2*: resilience to future conditions), or an individual that *suffers* (*Genotype 3*: energetic demands in excess of available ATP). Individuals of *Genotype 3* are not viable under the environmental change depicted.

rates (respiration is represented by the size of each pie-chart in Fig. 4).

This simplified scheme (Fig. 4) can be expanded to a wide spectrum of genetically determined responses to global change. Such differential responses at the individual level could have a major impact on population structure. The genotype that *suffers* may survive, if the stress is transient, but would not be able to survive under sustained environmental change. Other genotypes with limited available ATP could not respond metabolically to multiple stressors (e.g., temperature, acidification of the ocean, and lack of food) and would also be eliminated from the population. Such physiological insights are necessary for better predictions of Darwinian fitness (winners and losers) in a changing environment (Somero 2010).

A systems-biology approach: integrating genetics, genomics, and physiology

As originally defined by Ideker et al. (2001), "Systems biology studies biological systems by systematically perturbing them (biologically, genetically, or chemically); monitoring the gene, protein, and informational pathway responses; integrating these data; and ultimately, formulating mathematical models that describe the structure of the system and its response to individual perturbations." Ability to perturb complex biological systems comes, in turn, from a fusion of discovery-science and hypothesis-driven science. The former seeks to catalog all elements in the system and to engage in global analyses; the latter attempts to test specific mechanisms explaining organismal function. A necessary pre-requisite for systems biology is a genome sequence, although sufficient conditions for systems biology encompass everything from experimental tractability to traditional genetic and physiological methods, as described above, to application of other global, "-omic" tools and the capability for forward genetics (phenotype to genotype; see "Analysis of QTL" section) and reverse genetics (genotype to phenotype; see the "Proof of function: a major challenge" section). The scale of requirements for systems biology greatly limits the number of species amenable to this approach.

Genomics must be coupled with genetics

With respect to global problems of changing oceans, there are a growing number of marine animals with published draft genome sequences. For example, genomes are available for two species of the sea squirt *Ciona* (Dehal et al. 2002; Vinson et al. 2005), the

pufferfish *Takifugu rubripes* (Aparicio et al. 2002), the purple sea urchin *Strongylocentrotus purpuratus* (Sodergren et al. 2006), the Pacific oyster *C. gigas* (Zhang et al. 2012), the Atlantic cod *Gadus morhua* (Star et al. 2011), the owl limpet *Lottia gigantea*, a marine polychaete *Capitella teleta* (Simakov et al. 2013), the sea lamprey *Petromyzon marinus* (Smith et al. 2013), and the ctenophore *Mnemiopsis leidyi* (Ryan et al. 2013).

A genome sequence for an organism lacking methods for genetic analysis is, however, of limited use for elucidating the systems biology of response to environmental change, because genetic perturbations of the response are greatly constrained. Trans-generational populations and controlled crosses can be made with the purple sea urchin (Leahy et al. 1994; Cameron et al. 1999), the Pacific oyster (Hedgecock et al. 1995; Pace et al. 2006; Hedgecock and Davis 2007), the sea squirt (Hendrickson et al. 2004), and the ctenophore (Pang and Martindale 2009). An inbred female produced by four generations of brother–sister matings was used in the genome-sequencing project on oysters in order to reduce high levels of polymorphism, thus simplifying assembly of the genome (Zhang et al. 2012). Crosses of pedigreed lines of the Pacific oyster produce contrasting growth, survivorship, and physiological and biochemical phenotypes (Hedgecock et al. 1995; Pace et al. 2006; Hedgecock and Davis 2007; Curole et al. 2010; Meyer and Manahan 2010; Plough and Hedgecock 2011). Selected crosses of specific lines now reproducibly provide the biological perturbation required for understanding mechanisms of response.

Other "-omic" approaches generate hypotheses

A systems-biology approach is enabled by rapidly advancing technologies for transcriptomic, proteomic, and metabolomic profiling, which permit a broader, more in-depth, and precise description of phenotype. For example, we made one of the first in-depth analyses of the transcriptome of a marine metazoan so as to shed light on the functional genomic basis of genetically determined variation in larval growth of the Pacific oyster (Hedgecock et al. 2007). As the costs of sequencing nucleic acids have decreased, many researchers are now using high-throughput transcriptome profiling (e.g., RNA-Seq; the review by Wang et al. [2009] had 1593 citations in the Web of Science as of January 2014). These dramatic advances in the technology for sequencing nucleic acids are providing unprecedented abilities to quantify gene expression and abundances of mRNA, in particular for "non-model" organisms that are of interest to comparative biologists. Major advantages of RNA-Seq are that it does not require (but certainly benefits from) previous genomic or genetic resources and it enables a complete analysis of gene-expression responses to environmental perturbation. Still, RNA-Seq by itself is a hypothesis-generating, not a hypothesis-testing tool. Although results from RNA-Seq can and should be validated by biological and technical replication and by alternative methods (such as quantitative polymerase chain reaction), the mechanisms responsible for changes in gene expression and the presumed changes in physiology, which RNA-Seq may signal, remain hidden, especially in non-model systems. Similar advantages and disadvantages pertain to proteomic (Aebersold and Mann 2003) and metabolomic (Fiehn 2002) methods. These post-genomic tools are necessary but, by themselves, insufficient for elucidating the systems biology of response to global environmental change.

Genotype-to-phenotype mapping

A grand challenge of employing systems-biology to understand the complex responses of organisms to environmental change—the mapping of genotypic-to-phenotypic variation—is shared by efforts to understand the genomic bases of human diseases and agricultural production (Benfey and Mitchell-Olds 2008). A primary step in this approach is to identify regions of the genome controlling variance in complex traits—that is, quantitative trait loci (QTL). QTL can be determined by genome-wide association studies or by means of controlled crosses. The former requires either a pedigreed population or a case–control design and sample sizes in the thousands or tens of thousands for effective prediction of the risk of disease or of breeding values (Wellcome Trust Case Control Consortium 2007; VanRaden et al. 2009; Speliotes et al. 2010). The latter requires genetically enabled experimental systems and well defined, segregating phenotypes (e.g., Schadt et al. 2003).

Analysis of quantitative trait loci

We have used the experimental forward genetics approach, conducting QTL-mapping studies in F_2 populations of the Pacific oyster, to determine the genetic basis for various quantitative traits, including larval viability. For this trait, there is no measured phenotype, as there would be if one were mapping QTL for growth or size-at-age. Instead the phenotype is modeled as an unobservable liability reflected by

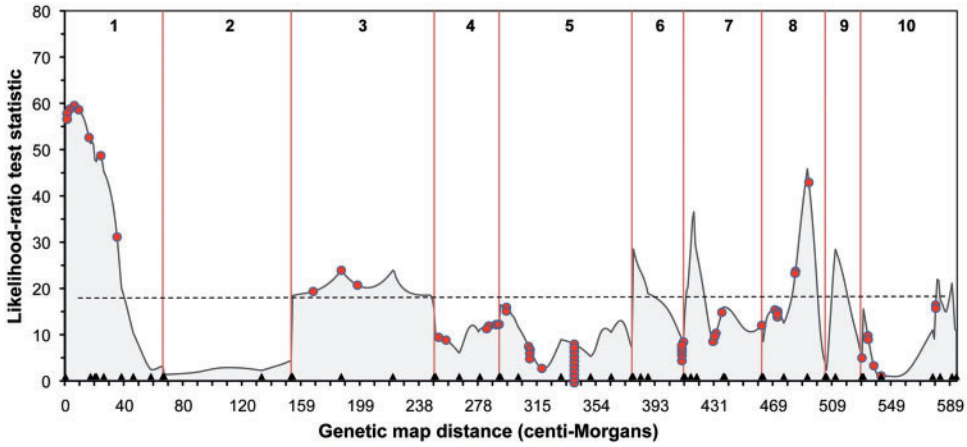

Fig. 5 Genotypic-to-phenotypic mapping, using QTL analysis in an F_2 population of the Pacific oyster *C. gigas*. For each unit across the genetic map (*x*-axis), the plot gives the likelihood-ratio test statistic (*y*-axis) for distortions of Mendelian ratios, owing to viability selection. Long vertical lines mark the ends of linkage groups, numbered 1–10 (*C. gigas* has 10 chromosomes); small triangles along the *x*-axis indicate the position of markers used in the mapping procedure; dashed horizontal line indicates the genome-wide threshold value for significance (modified from Plough and Hedgecock [2011] with the permission of the Genetics Society of America). Overlaid on the QTL trace are the genomic locations of 58 genes coding for cytochrome P450 (CYP, circles; D. Hedgecock, unpublished data) proteins, illustrating how, under an hypothesis that CYP proteins might be associated with mortality, most *CYP* genes could be eliminated and only a few retained as causal candidates.

deviations from Mendelian expectations of genotypic proportions at linked, observed markers (Luo et al. 2005). QTL results are typically displayed as a trace of a likelihood-ratio test statistic for association between genotype and phenotype across the genome (Plough and Hedgecock 2011). Peaks in the QTL trace (Fig. 5) indicate regions of the genome in which a statistically significant genotype-to-phenotype correspondence exists; again, in the case of viability, peaks represent regions of the genome in which genotype-dependent mortality (i.e., viability selection) prior to sampling has altered genotypic proportions. QTL-mapping thus localizes the causes of mortality, permitting focus on particular regions of the genome that are on the order of 10 genetic map units (centiMorgans or cM) in length (the width of the statistical confidence interval around the QTL peak). Background mortality can be distinguished from environment-specific mortality by comparing the QTL profiles of the same family reared under control and treatment conditions (Plough 2012).

Given the correspondence of physical and genetic maps in model species and the oyster (1 cM $\approx$ 1 million base pairs) and the densities of genes observed in the oyster, that is, 28,072 predicted genes in a total genome of 637 million base pairs (Zhang et al. 2012), each QTL peak may correspond to ~440 genes, on average. The search for causal genes can be narrowed further by combining information on functional annotation of genes with results from gene-expression analyses and from temporal studies of patterns of mortality (Plough and Hedgecock 2011).

Much of the QTL-mapping in the Pacific oyster to date has been conducted on juveniles, although individual larvae can be genotyped for a modest number of markers (see Plough and Hedgecock 2011). A significant challenge going forward is to measure allocations of energy and to type markers on a genomic scale for individual larvae, enabling whole-genome mapping of organismal physiology.

Narrowing the focus: wading through genomic redundancy

One of the surprises to emerge from the genome sequence of the Pacific oyster, in comparison to other genomes, is the large number of genes involved in major stress–response pathways and the high level of redundancy of gene families responding to biotic and abiotic environmental stress (Zhang et al. 2012). For example, the oyster has 88 genes for heat-shock protein 70, compared with only ~17 in humans, and 133 genes coding for cytochrome P450 (CYP) proteins, compared with only ~65 in humans. In the search for causal physiological mechanisms of response to stress, such redundancy poses a major challenge. Nevertheless, QTL-mapping in a genetically enabled model species offers one means for cutting through such redundancy, as illustrated by overlaying the locations of genome scaffolds containing mapped single-nucleotide polymorphisms and 58

CYP genes onto the viability QTL map (Fig. 5). If certain *CYP* genes are causal candidates for survivorship, then those *CYP* genes lying outside of QTL peaks could be eliminated from further consideration. Physiological analyses could be more narrowly focused on the few genes associated with significant QTL peaks. For studies of the physiology of stability and change, the approach of integrating physiology, genetics, and genomics offers a novel way forward.

Proof of function: a major challenge

After identifying candidate genes using forward genetics (Fig. 5), a critical step is to characterize their function. This can be achieved using the approaches of reverse genetics that seek to determine the phenotypes that arise as the product of specific, known DNA sequences. A variety of techniques may be employed to increase, decrease, or eliminate the expression of candidate genes *in vivo* (knockout and knockdown), or in isolated cell culture (heterologous expression). For example, we have been successful in applying heterologous-expression techniques to characterize amino acid transporter genes in a sea urchin (Meyer and Manahan 2009). In that study, mRNA transcribed from genes putatively encoding transporters for amino acids, when microinjected into a heterologous-expression system (the oocytes of *Xenopus laevis*), increased the transport of alanine 40-fold. A great deal more work needs to be done in this area of proof of function, as there is a growing imbalance between the extensive lists of candidate genes emerging from ''-omic'' studies and the paucity of direct tests of function.

Concluding perspective

Experimental challenges, using wild-type animals to study responses to global environmental change, frequently produce results that suggest a reduction in mean population fitness. However, these experiments have rarely employed approaches that address potentially adaptive, genetic variation within populations. Well-conceived experiments that manipulate genotype and environment to produce phenotypic contrasts and that integrate genomic, transcriptomic, proteomic, and metabolomic approaches with classical biochemical and physiological approaches are going to be required to understand and to predict the Darwinian biological responses of organisms to global change. Such a systems-biology approach must focus on model species with well-developed genetic, genomic, and physiological resources. There are no perfect model systems, but we have suggested at least some of the properties of a model marine species (high fecundity, complete life-cycle culture, well-developed genetic, genomic, and physiological resources) and argued further for a focus on developing larval stages, which enable an organismal approach. Our model, the Pacific oyster *C. gigas*, has many of these features, more than most marine metazoans, but still presents challenges for simultaneous forward genetic and physiological analyses of individual larvae and requires further development of methods for reverse genetics.

The research program that we have described provides mechanistic insight into how organisms may respond to the environmental stresses expected with global anthropogenic changes. A strong emphasis in this program is on elucidating standing genetic variation in the way that energy is allocated to various physiological processes. Much of the existing work attempts to assess biological responses to environmental change, in traits such as survival, growth rate, morphology, and gene expression. Although the possibility of increased demand for metabolic energy is often inferred, actual expenditure of energy rarely is measured. We need to know the extent to which biological responses to compounding environmental changes will require complex tradeoffs in allocation of ATP between the routine cost of living and additional costs of stress. If demands exceed the ATP pool (Fig. 4; Genotype 3 *suffers*), the resulting energetic insufficiency may not be immediately apparent at the level of the whole-organism but will have important long-term consequences for fitness traits.

Still, the question remains how such reductionist ''bench science'' can be applied to predict ecosystem responses. A start on this complex problem could be achieved through partnerships with mathematical ecologists and modelers, who are adept with individual-based models (Grimm and Railsback 2005) that incorporate detailed oceanographic and climatic models and can simulate future environments (e.g., Hofmann et al. 2006). Results from the experimental approach outlined here could inform such models with realistic biological mechanisms and Darwinian variability, providing greater insight into how, and if, animal populations may persist under conditions of global environmental change.

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