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REVIEW

Mosaic physiology from developmental noise: within-organism physiological diversity as an alternative to phenotypic plasticity and phenotypic flexibility

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ABSTRACT

A key problem in organismal biology is to explain the origins of functional diversity. In the context of organismal biology, functional diversity describes the set of phenotypes, across scales of biological organization and through time, that a single genotype, or genome, or organism, can produce. Functional diversity encompasses many phenomena: differences in cell types within organisms; physiological and morphological differences among tissues and organs; differences in performance; morphological shifts in external phenotype; and changes in behavior. How can single genomes produce so many different phenotypes? Modern biology proposes two general mechanisms. The first is developmental programs, by which single cells and their single genomes diversify, via relatively deterministic processes, into the sets of cell types, tissues and organs that we see in most multicellular organisms. The second general mechanism is phenotypic modification stemming from interactions between organisms and their environments – modifications known either as phenotypic plasticity or as phenotypic flexibility, depending on the time scale of the response and the degree of reversibility. These two diversity-generating mechanisms are related because phenotypic modifications may sometimes arise as a consequence of environments influencing developmental programs. Here, I propose that functional diversity also arises via a third fundamental mechanism: stochastic developmental events giving rise to mosaics of physiological diversity within individual organisms. In biological systems, stochasticity stems from the inherently random actions of small numbers of molecules interacting with one another. Although stochastic effects occur in many biological contexts, available evidence suggests that they can be especially important in gene networks, specifically as a consequence of low transcript numbers in individual cells. I briefly review known mechanisms by which organisms control such stochasticity, and how they may use it to create adaptive functional diversity. I then fold this idea into modern thinking on phenotypic plasticity and flexibility, proposing that multicellular organisms exhibit 'mosaic physiology'. Mosaic physiology refers to sets of diversified phenotypes, within individual organisms, that carry out related functions at the same time, but that are distributed in space. Mosaic physiology arises from stochasticity-driven differentiation of cells, early during cell diversification, which is then amplified by cell division and growth into macroscopic phenotypic modules (cells, tissues, organs) making up the physiological systems of later life stages. Mosaic physiology provides a set of standing, diversified phenotypes, within single organisms, that raise the likelihood of the organism coping well with novel environmental challenges. These diversified phenotypes can be distinct, akin to polyphenisms at the organismal level; or they can be continuously distributed, creating a kind of standing, simultaneously expressed reaction norm of physiological capacities.

KEY WORDS: Stress, Tradeoff, Costs, Stochasticity, Sampling error, Physiological systems, Noise, Homeostasis, Evolution, Development, Functional diversity, Cell size, Multicellularity, Canalization, Ergodic principle

Introduction

A defining characteristic of multicellular organisms is functional diversity. Functional diversity among individuals is obvious, almost regardless of relatedness: it's easy to see when comparing individuals from one species with those from another, and it's almost as easy to see among individuals from within given populations. Less obvious are the origins of those differences, and over the past 150 years this problem has attracted significant attention from geneticists and evolutionary biologists (Darwin, 1859; Dobzhansky, 1937; Mayr, 1963; West-Eberhard, 2003).

The fact of functional diversity within individuals, the topic of this paper, is obvious too, although it can be less apparent to the casual observer. Intra-individual diversity appears in two ways. The first is spatial: individuals have sets of modules – cells, tissues and organs – that differ from one another chemically, morphologically and physiologically. This kind of diversity is simultaneous but spatially distributed; in effect, organisms exhibit multiple lower-level phenotypes at the same time but in different places. Such modular diversity provides key divisions of labor, and it expands the range of organismal capacities. These effects likely played important roles in the evolutionary origins of multicellular organisms from unicellular ancestors (Bonner, 2000; Buss, 1987; Knoll, 2011).

The second way functional diversity appears within individuals is temporal: individuals have different phenotypes at different times in their lives. Clearly, phenotypes of individuals change as they age. Adults usually don't look, act or function like they did when they were embryos or juveniles, and the collective set of changes over a lifetime describes the organism's ontogenetic trajectory. Individuals also dynamically alter their phenotypes in response to environments they encounter, which can differ profoundly from one individual to the next, even if those individuals start out with similar genotypes and with similar maternal and epigenetic legacies. The classification of such temporal effects has been contentious (Debat and David, 2001), and here I will simply use the terms phenotypic plasticity (Bradshaw, 1965; Pigliucci, 2001; West-Eberhard, 2003) and phenotypic flexibility (Piersma and Lindström, 1997; Piersma and van Gils, 2010). Phenotypic plasticity refers to genotype-specific changes in phenotype as functions of the developmental environment, and these changes usually are irreversible. By

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contrast, phenotypic flexibility specifies more labile and reversible changes of phenotype in response to new environments.

A first synthetic claim, which I state, justify briefly and then use as a bridge to what follows: most functional diversity within organisms emerges from deterministic processes. That is, functional diversity emerges under the direction of identifiable actors – genes, the networks they form, the differentiated cells they produce, and the physiological processes those networks build. For example, the diversity of cell types, arising in animals during development, comes from stereotyped interactions among gene networks interacting within and among cells and tissues, and from the interaction of those networks with stereotyped sets of physical and chemical conditions inside and outside the embryo (MacNeil and Walhout, 2011). Likewise, the functional (phenotypic) diversity generated by phenotypic plasticity reflects evolved, repeatable and sometimes stereotyped responses of developing organisms to differences in the environments they experience; the reaction norm is a meta-property of the networks and physiology underlying development. Likewise for phenotypic flexibility: organisms reversibly adjust their physiology and behavior, but those changes emerge from pre-built sensory and physiological systems, which themselves reflect something more deterministic built from genetic and epigenetic actors. I am not suggesting that all functional diversity is deterministic (Losick and Desplan, 2008), nor do I think that others think so. Similarly, I do not claim that the environment itself varies in deterministic ways when it drives plasticity and flexibility. Nevertheless, the prevailing view of the mechanistic origins of diversity is largely deterministic.

Below, I propose an alternative explanation of the origins of intra-individual functional diversity – an alternative that invokes stochasticity. I first discuss the biology of stochasticity, drawing on several important reviews from the past 15 years (Kærn et al., 2005; Kilfoil et al., 2009; Losick and Desplan, 2008; MacNeil and Walhout, 2011; McAdams and Arkin, 1999; Paulsson, 2004; Raj and van Oudenaarden, 2008; Rao et al., 2002), including brief summaries of how organisms control stochastic noise and how, in some circumstances, they may use it to their benefit. Building from ideas discussed recently by MacNeil and Walhout (MacNeil and Walhout, 2011), I then propose that stochastic events in cells can, via development, generate functional diversity at higher levels of biological organization within multicellular organisms. This functional diversity constitutes a new kind of phenotypic, or functional, diversity, which I call ‘mosaic physiology’. Mosaic physiology describe simultaneous, spatially distributed diversity in cell- and tissue-level functions arising from stochastic processes, and I propose that it plays roles in the ecology and evolution of organisms that are just as important as the roles imagined both for deterministic differentiation during the developmental program and for the environmentally dependent phenotypes described by phenotypic plasticity and flexibility (Bradshaw, 1965; Ghalambor et al., 2007; Piersma and Drent, 2003; Pigliucci, 2001).

Noise and the physiology of finite numbers

Physiological noise can be partitioned into two kinds: extrinsic and intrinsic (Blomberg, 2006; Horsthemke et al., 1992). The first arises from variation in physiological factors normally under tight homeostatic control, which usually is driven by variation in some environmental (extrinsic) factor. The main physiological factors under control have been known since the late 19th and early 20th centuries (Bernard, 1865; Bernard, 1878; Cannon, 1929; Cannon, 1932) and include pH, osmolality, concentrations of calcium and glucose, and others. Their control has been studied intensively because they are so important to human health and to organismal performance generally. A recent paper (Woods and Wilson, 2013) cast variation in these factors as key sources of global physiological noise – in the sense that variation in the factors has strong and inescapable effects on all other physiological parts and communication systems in organisms. In this view, physiological homeostasis is a way of reducing the background noise so that intra-individual communication of all kinds is faster and less prone to error.

The second source, intrinsic noise – the focus of this paper – stems from the molecular stochasticity that accompanies processes containing small numbers of entities, also known as finite-number effects (Kærn et al., 2005). Our knowledge of these effects can be traced to the classic work of Erwin Schrödinger. In his book *What is Life?*, Schrödinger (Schrödinger, 1944) asked: how small could a living thing be? His answer was framed in terms of how many atoms or molecules the smallest living entity could contain. He concluded that there was a lower limit: that living entities must be large enough so that statistical noise, arising from the random walks taken and collisions experienced by individual atoms and molecules, disappears into the net behaviors of large ensembles. In Schrödinger’s words (Schrödinger, 1944), ‘An organism must have a comparatively gross structure in order to enjoy the benefit of fairly accurate laws, both for its internal life and for its interplay with the external world.’ In other words, determinism arises from large numbers, implying conversely that small numbers can erode it. The argument below is a physiological generalization of Schrödinger’s.

Physiologists often think of organisms as having continuous, analog traits, because we measure concentrations, voltages, rates of transcription or flow, etc. But organisms and their components depend on finite numbers of discrete entities, including individual free protons, ions, proteins, transcription factors, etc. In this sense, organisms are digital, and noise in such systems can be stochastic. The distinction between digital and analog physiology reflects, in part, Schrödinger’s observations on the statistics governing ensembles of events, in that physiological functions that seem analog are supported by a large number of digital events, in the same way that digital music sounds smooth (analog) even though it is the net outcome of many fast binary events, or that a binomial distribution becomes normal as the number of trials becomes large. In addition to being digital, physiology can also be viewed as binary: abstracted, physiological processes mostly concern entities in either of two states, such that we can represent analog states as collections of binary digits (bits). For example, the main energy currency in organisms – adenosine and its coupled phosphate groups – takes on high- or low-energy forms (ATP and ADP, respectively). These states could be represented by ones and zeros (bits). Ligands and transcription factors are free or bound, again binary. Likewise, one could describe ions in analog terms – as concentrations, or osmotic pressures, or potential differences across membranes. But those same analog traits can be recast as ions existing on one side of a membrane or the other, a binary distinction. At higher levels of control, in gene regulatory circuits for example, the directionality can be reversed: analog inputs can be converted to binary outputs (all or none rates of transcription) by positive feedback (Becskei et al., 2001).

There are reasonable similarities between the behaviors of physiological systems containing small numbers of particles and the statistics of small sample sizes (Liao et al., 2012a). Here, I examine these similarities both by simulating binomial distributions and then by constructing and analyzing a somewhat more complicated, and

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perhaps more realistic, simulation of ligands interacting with receptors.

It is intuitive that parameter estimates improve as we sample more data. Although the statement implies 'an observer', it could equally well describe a physical or physiological situation in which one kind of device or system interacts with some other particle or chemical. Schrödinger (Schrödinger, 1944) wrote: 'If I tell you that a certain gas under certain conditions of pressure and temperature has a certain density, and if I expressed this by saying that within a certain volume (of a size relevant for some experiment) there are under these conditions just N molecules of the gas, then you might be sure that if you could test my statement in a particular moment of time, you would find it inaccurate, the departure being on the order of $\sqrt{N}$. Hence if the number N=100, you would find a departure of about 10, thus relative error=10%. But if =1 million, you would be likely to find a departure of about 1000, thus relative error=1/10%.' This kind of sampling error has been discussed more recently by others (Kærn et al., 2005; Thattai and van Oudenaarden, 2001). Another way to examine this idea is by sampling simulated data, which I do for a binomial process in Fig. 1. In this example, the error in estimating the true binomial probability (P=0.5) was very large when observing fewer than 10 events and was non-trivial even for 100.

The examples above could be rejected on the grounds that physiology is more complicated than gas molecules in defined volumes, or than coin flipping and other binomial processes. To examine finite-number effects in a somewhat more realistic context, I simulated ligands interacting with receptors using the Smoldyn software package (http://www.smoldyn.org/) (Andrews et al., 2010) (Fig. 2). The fluctuations over time, in the fraction of receptors occupied, depended on the size of the space, the diffusion coefficients of the ligands, and the probabilities of binding and unbinding. However, the qualitative pattern turned out as expected: the probability densities of the fraction of receptors occupied (a graphical view of the sample variance) were substantially broader when there were 24 ligands and 24 receptors, compared with when there were 100 or 1000 of each. Qualitatively similar results were obtained from a different simulation model by Kærn and colleagues (Kærn et al., 2005). The caveats to such an approach are many: reactions can occur in macromolecular complexes, in which substrates and products are handed sequentially from one reaction to the next (Ovádi and Srere, 2000; Srere, 1981); macromolecular crowding and compartmentalization can strongly affect molecular movements and reaction dynamics (Ellis, 2001; Turner et al., 2004; Zhou et al., 2008); and noise can propagate in interesting ways through signal cascades involving small numbers of molecules (Morishita et al., 2006; Thattai and van Oudenaarden, 2001). Nevertheless, the simulation in Fig. 2 captures the magnitude of the potential stochasticity problem.

In real physiological systems, do small numbers give rise to stochasticity? Increasingly, across a range of systems, we know that the answer is 'yes'. As Bialek (Bialek, 2012) summarizes in his book on biophysics, many systems operate close to the limits allowed by basic physics and chemistry – because they are using, or responding to, individual molecular events: human retinal cells and fly ommatidia can produce distinct electrical responses to single photons, individual transcripts in cells can occur in one or a few copies, bacteria can count individual molecules during the process of chemotaxis, and proofreading machinery steps through DNA base pair by base pair.

For my purposes below, however, the key problem is to determine whether cells, and more generally, development, show observable

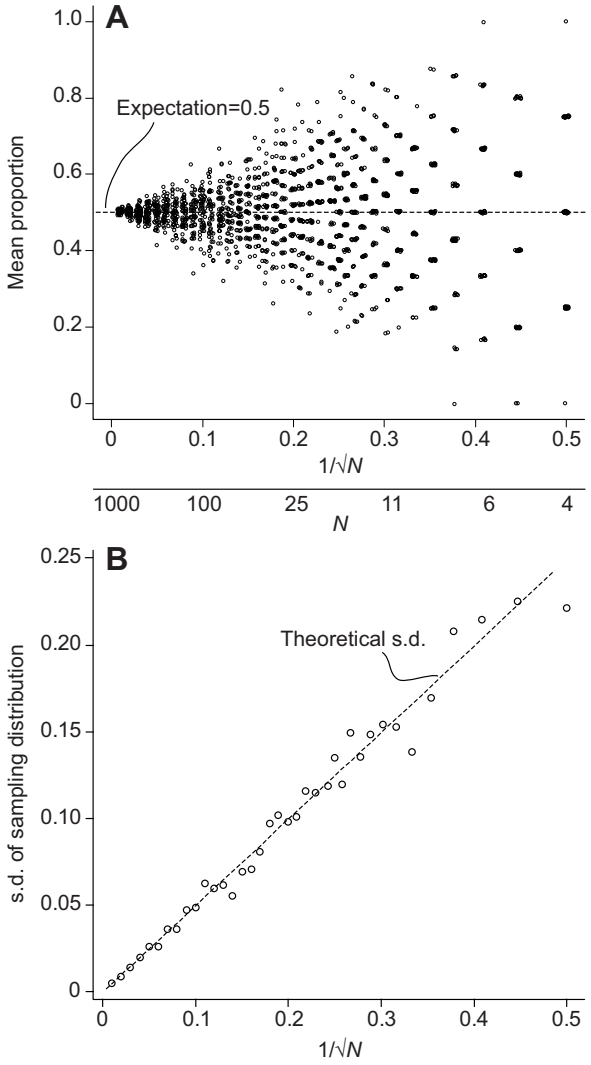

Fig. 1. Error from small numbers. (A) Variation in binomial sampling distribution as a function of the number of observations. Each data point is the mean of N observations drawn from a binomial distribution with P=0.5, and for each value of N the 'experiment' was replicated 50 times. To avoid complete over-plotting of points, they were jittered in both x and y directions. Less variation was expected in the sampling distribution as the number of observations increased, and indeed this was the case. (B) The standard deviation of the simulated data ($\sigma_{\hat{p}}$), plotted as a function of $1/\sqrt{N}$, closely matches the expected value shown by the dashed line (i.e. the dashed line is not a fit to the simulated data, but a theoretical expectation). The equation describing the expected value of the sampling distribution is $\sigma_{\hat{p}}=\sqrt{[p(1-p)/N]}$.

stochasticity. I frame this problem as two smaller questions, both focused on cells because there are relatively plentiful data available on cellular systems. First, within single cells, how few particles actually interact? Second, do cells show observable stochasticity? Answering the first question is straightforward for some biological molecules (transcripts, proteins) but not others (microcomponents of the membrane and cytoplasm). The second question has also been answered, in the affirmative, by a large set of empirical studies, discussed below.

In cell systems, how few particles interact? Generally biologists do not count ions or molecules directly but infer them from their effects [there are exceptions; e.g. it is possible to count mRNA transcripts directly (Raj et al., 2006)]. I analyze this problem with

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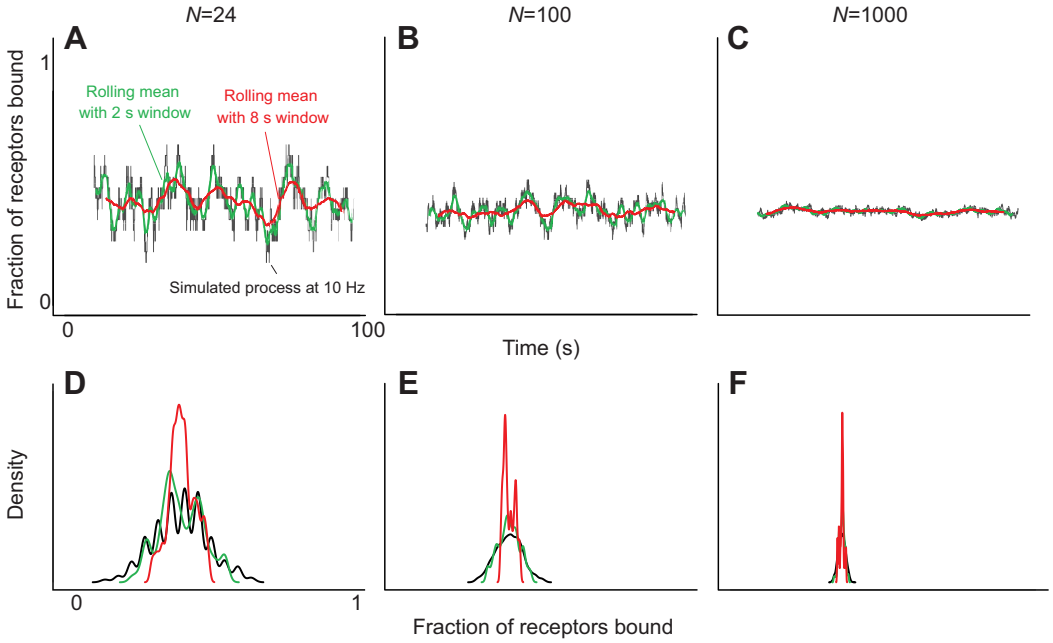

Fig. 2. Simulation of ligand–receptor interactions in a three-dimensional space. Simulations were run using Smoldyn (Andrews et al., 2010). In all panels, the diffusion and reaction kinetics were identical, and the receptors and receptor–ligand complexes were bound to the walls of the simulated volume (a cube). Ligands underwent isotropic diffusion in the simulated space and were reflected whenever they struck a wall. What varies left to right is the total number of ligands (=number of receptors): 24 in A, 100 in B and 1000 in C. In addition, the volume of the simulated space was scaled to the number of ligands so that ligand density was constant. The green line represents the rolling mean calculated using a window 2 s wide and the red line the rolling mean with a window 8 s wide. D–F show density plots associated with the simulations in A–C. In all cases, the first 12 s of the simulation was omitted, because there were artifacts arising from the initial conditions.

reference to the average number of free protons in a single cell of *Escherichia coli*. Assuming a cytoplasmic pH of 7, there are 10^{-7} moles of free protons per liter, or about 6×10^{16} protons per liter. A typical *E. coli* is cylindrical with a radius of about 0.5 μm and length 2.5 μm, which gives it a volume of 2 μm^3 ($=2\times10^{-15}$ l). Thus, in a single cell, there are only about 118 ($=6\times10^{16}$ protons $l^{-1}\times 2\times10^{-15}$ l) free protons at any instant. This number lies near the noise cut-off suggested by the simple simulations above. More generally, this calculation suggests that any biological compound less concentrated than 10^{-7} mol l^{-1} (=100 nmol l^{-1}), occurring in a cell the size of *E. coli* – or of higher concentrations in cells smaller than *E. coli* – may exist in a range where stochastic noise starts to become important. The key caveat is that the frequency with which those compounds interact with other compounds matters a lot, a point to which I return in the next section. In fact, many biological molecules occur at lower concentrations (McAdams and Arkin, 1999; Wodicka et al., 1997) (see Table 1). For example, more than 80% of the genes on the chromosome of *E. coli* produce fewer than 100 copies per cell of their protein products (Guptasarma, 1995). Likewise, in a eukaryote, protein copy numbers in the yeast *Saccharomyces cerevisiae* range from <50 to more than 1 million (Ghaemmaghami et al., 2003). Transcript levels are even lower, with most expressed genes having expression levels of, on average, 1 or fewer copies per cell (Holland, 2002; Wodicka et al., 1997).

Furthermore, *E. coli* is not particularly small. In a survey of bacteria naturally occurring in seawater, Lee and Furhman (Lee and Fuhrman, 1987) found cell volumes of 0.036 to 0.073 μm^3 (=femtoliters, fl), which is 3.6×10^{-17} to 7.3×10^{-17} l, or nearly 100-fold smaller than *E. coli*. Based on protein copy numbers in *E. coli*, these small marine cells must contain many proteins with copy numbers in the single digits. By comparison, cells of vertebrates generally are much larger. Volumes of nucleated erythrocytes from 159 species of vertebrates had volumes of 100–10,000 μm^3 (Gregory, 2001) and, in mammals specifically, volumes of 20–3000 μm^3 (Savage et al., 2007). These large cells are therefore much less likely to contain very small numbers of proteins or other molecules (Raj and van Oudenaarden, 2008), although slow diffusion, compartmentalization and macromolecular crowding can subdivide the spaces into domains where finite-number effects may loom large (van Zon et al., 2006; Zhou et al., 2008).

The second question, whether cellular stochasticity has been confirmed empirically, has an easy answer: it is extremely common and perhaps ubiquitous (Balázsi et al., 2011). Table 1 summarizes a non-exhaustive set of examples showing stochasticity in the number of molecules and in cell phenotypes. Collectively, the examples can be characterized in three ways. The first is that almost all involve differences between cells, rather than between entities at higher or lower levels of organization. Second, stochasticity in cell phenotypes appears to arise from noise in gene-regulatory networks, or in the machinery that produces and destroys transcripts and proteins (Newman et al., 2006; Salari et al., 2012), but not from noise in non-genetic components. Third, in studies of multicellular organisms, stochasticity in cell states largely reflects developmental switching: early regulatory stochasticity sends cells down one of several pathways of differentiation, after which their states are fixed.

Such a gene-centered view differs substantially from the foundational ideas of Schrödinger (Schrödinger, 1944), whose argument invoked biological particles generally, and it differs from the finite-protons example developed above. This mismatch reflects either of two interesting possibilities. One is that transcription factors, transcripts and proteins are the only biological molecules rare enough to show finite-number effects. Perhaps most other cell components – protons, ions, lipids, ATP, neurotransmitters, etc. – are so numerous that their individual actions disappear completely into

Table 1. Selected examples of finite numbers and phenotypic stochasticity in biological systems

Reference	Taxon	Result
Unicellular		
Banerjee et al., 2004	Bacteria	*Lac* operon state fluctuations across bacterial life cycle.
Becskei and Serrano, 2000	Bacteria	Constructed gene circuits with negative feedback show lower noise in transcription factors.
Guptasarma, 1995	Bacteria	Over 80% of *E. coli* genes expressed at <100 copies of protein products per cell.
Elowitz et al., 2002	Bacteria	Variation in single-gene expression; contributions of both intrinsic and extrinsic noise.
Isaacs et al., 2003	Bacteria	Noise in gene autoregulatory networks necessary for reproducing observed distributions of phenotypes.
Korobkova et al., 2004	Bacteria	Noise in the intracellular networks generates behavioral noise in single-cell chemotaxis.
Ozbudak et al., 2002	Bacteria	Significant phenotypic noise in gfp-linked proteins in populations of *Bacillus subtilis*.
Acar et al., 2008	Yeast	Stochastic switching among phenotypes enhances population growth in variable environments.
Becskei et al., 2005	Yeast	Significant noise introduced by low-frequency, random gene activation.
Blake et al., 2003	Yeast	Different levels of noise associated with transcriptional efficiency and control; increased noise can lead to bi-stable output phenotypes in cells.
Blake et al., 2006	Yeast	Higher noise in protein levels permits populations to perform better during extreme environmental stress.
Colman-Lerner et al., 2005	Yeast	Large variation in phenotypic response to mating pheromone; most variation associated with pathway capacity and expression capacity, not expression noise.
Ghaemmaghami et al., 2003	Yeast	Yeast proteome contains proteins in numbers ranging from 50 to 1,000,000 per cell.
Holland, 2002	Yeast	Many mRNAs present on average at <1 copy per cell.
Newman et al., 2006	Yeast	Over 2500 proteins assessed on cell-by-cell basis; significant differences in noise based on mode of transcription and gene function.
Raser and O'Shea, 2004	Yeast	Gene-specific, noisy differences in expression between alleles at the same locus.
Wodicka et al., 1997	Yeast	Expression levels range from 0.1 to several hundred copies per cell; 50% <1 copy per cell.
Multicellular		
Bengtsson et al., 2005	Mice	Stochastic gene expression in mouse pancreatic cells.
Boettiger and Levine, 2009	Flies	Stochastic and synchronous gene activation in *Drosophila* embryos depending on pre-loading by RNA polymerase II.
Chang et al., 2008	Mouse	In blood stem cells, spontaneous outliers containing a stem cell marker shift distributions of cell types; differences affect subsequent cell fate.
Jimenez-Gomez et al., 2011	*Arabidopsis*	Quantitative trait loci that control variation in stochastic noise of glucosinolates.
Perc et al., 2009	Mice	Stochasticity in response of pancreas cells (in tissue slices) to acetylcholine.
Raj et al., 2006	Hamsters	Massive variation in total number of mRNA molecules in clonal ovary cells.
Raj et al., 2010	Nematodes	Stochastic variation in expression patterns in intestinal cells of mutant *C. elegans*, caused by loss of network elements.
Sigal et al., 2006	Humans	Long-term stochastic changes in protein levels in populations of lung carcinoma cell line.
Tsuboi et al., 1999	Mice	In sensory neurons, random but mutually exclusive expression of different odorant receptors.
Wernet et al., 2006	Flies	Stochastic expression of Spineless drives random expression of photoreceptors with different color receptors.

the statistics of large ensembles. Alternatively, perhaps these other components are also stochastic, but we (biologists) have not had the tools for looking at them, or the interest in doing so. Finally, the studies so far on animals, with their bodies divided into germline and soma, indicate that stochastically driven variation in phenotypes in the soma reflects the developmental legacy of multicellular tissues having developed from just one or a few cells.

Controlling stochastic noise…

Stochastic noise in cells can be performance depressing, if not outright dangerous. Not surprisingly, cells have evolved diverse ways of controlling it (Balázsi et al., 2011). Noise dampening can be especially important early in development (of multicellular organisms), when the total numbers of cells and molecules are low, and the effects of noise may be amplified via cell lineage diversification and growth (Arias and Hayward, 2006; Balázsi et al., 2011). One of the most important mechanisms is negative feedback (Yu et al., 2008), which in eukaryotes can involve interactions between microRNAs derived from introns and the promoters of those genes (Singh, 2011). Other processes and factors that modulate intracellular noise include: redundancy in gene networks (McAdams and Arkin, 1999; Raj et al., 2010), interactions between proteins and other background molecules and perhaps macromolecular crowding generally (Morishita and Aihara, 2004), the dynamics of signaling cascades (Morishita et al., 2006), alignment of dose–response kinetics (Yu et al., 2008), communication and spatial averaging among cells or nuclei (Gregor et al., 2007; Tanouchi et al., 2008), and aspects of DNA macro-structure, such as looping structure (Vilar and Leibler, 2003), methylation patterns and chromatin composition (Bajić and Poyatos, 2012; Viñuelas et al., 2012).

There is another process, temporal averaging, which may be broadly applicable in biology, and which may explain why stochastic effects so far have been observed mostly in gene–protein networks. In temporal averaging, biological entities integrate more rapid interactions over time, a process that averages out noise in the rapid events (equivalent to experimenters reducing variance in their estimate of a mean value by taking more samples over time; also equivalent to sliding-window averaging over time, as exemplified by the green and red lines in Fig. 2). This is a biological manifestation of the 'ergodic principle' from physics, which posits that the behavior of a single particle over time is similar to the ensemble behavior of many particles at a particular moment (see Kapanidis and Strick, 2009) [for a different view, see Blomberg (Blomberg, 2006)]. In other words, just a few biological particles (or

molecules) whose activities are integrated over a long time, by slow-acting partners, are similar to many particles interacting with fast-acting partners over a short period of time. This principle underlies some modes of noise reduction in gene networks. For example, Kar and colleagues (Kar et al., 2009) modeled stochastic effects in the control system regulating the cell cycle in yeast, which is of particular interest because some cell cycle transcripts occur at about 1 copy per cell (see also Table 1). Such low copy numbers should inject enormous stochastic noise into the cell cycle. In their model, however, they did obtain stable cell cycling, but only when two key cell cycle transcripts had half-lives of <1 min. Such short transcript lifetimes in effect forced the translation machinery to average transcript numbers over time, resulting in significantly less noise than expected from transcript numbers alone.

The conclusion is that rapid interactions make up for small numbers, an effect that profoundly amends the finite-proton example above. Based on reasonable assumptions, I calculated that an average cell of *E. coli* contains about 118 free protons, a number so low that it should generate significant local noise in charge and acid–base status. However, protons interact with their surroundings enormously more rapidly than do proteins. One measure of this interaction speed is the diffusion coefficient, which for protons in water at 25°C is 762×10^{-7} cm^2 s^{-1} (Lee and Rasaiah, 2011), for lysozyme is 11.1×10^{-7} cm^2 s^{-1} (Brune and Kim, 1993) and for green fluorescent protein is 8.7×10^{-7} cm^2 s^{-1} (Swaminathan et al., 1997). Thus, the protein diffusion coefficients are 75–95 times lower, and the ratio may expand even more in cytoplasmic spaces crowded with macromolecules. The microscopic details of proton movement in water, and between acids and bases in water, are increasingly well known (Marx et al., 1999; Mohammed et al., 2005). Proton movements can be ultrafast, and may involve protons ‘hopping’ via water bridges or by the delocalization of local structural defects in hydrogen-bonded networks. These observations together suggest that a given proton has many more interactions with its macromolecular surroundings, per unit time, than does any given protein – i.e. the effects of a finite number of protons are spread out in space by their rapidity in time. This ergodic effect may be quite general, such that small cellular components (anything smaller than a small protein) both occur in larger numbers and have more rapid interactions. In this way, the ergodic effect reinforces the tendency of small particles not to produce stochasticity on time scales relevant to macromolecular processes in cells.

A final method of combatting noise may be larger size. All else being equal (a dubious assumption), larger cells will contain more of everything, which may partially mitigate small-numbers stochasticity. A useful way to conceive of the problem is in terms of counteracting evolutionary tendencies. The first is that evolution should push for smaller, simpler systems, because useless redundancy wastes materials and energy. But a key counterbalancing factor is statistical noise. If physiological systems (most often cells) become small enough, and depend on the actions of too few entities, statistical noise inherent in the systems will overwhelm their analog functions. This competing process should push for the evolution of larger, more redundant physiological systems. There are of course many other factors, including genome size, that affect the evolution of cell size (Gregory, 2001). However, the stochasticity problem is particularly stimulating, both because it is so general and because cell sizes range so widely (<0.1–10,000 fl), as summarized above, with eukaryotic cells generally being much larger than prokaryotic. This observation suggests that prokaryotic and eukaryotic cells differ fundamentally in their relationships to noise. Those differences can take on two forms – either prokaryotic cells are more tolerant of noise than are eukaryotic cells, or prokaryotic cells control noise more closely. This is an area ripe for scaling studies, which could examine the cell-size allometry of molecular feedback loops, transcriptional and translational dynamics, half-lives of transcripts and proteins, etc. In addition, it would be revealing to examine the correlated evolution of noise-control mechanisms in cell lineages that have evolved a larger or smaller size (Mongold and Lenski, 1996).

...but not too closely

The diversity of noise-reducing mechanisms discussed above implies that noise often depresses performance. Nevertheless, there are at least three broad reasons for organisms not to control noise too closely.

It costs too much

For organisms, positive action generally incurs costs. Homeostatic systems incur costs in the currencies of energy and materials devoted to constructing and maintaining tissues and organs. In addition, combatting one kind of noise may disrupt other homeostatic systems, via their linked information processing pathways. To combat intrinsic noise, organisms may contain greater numbers of larger cells, each with higher standing numbers of each component. Yet, such a mechanism may increase the energy and materials needed for support (Balázsi et al., 2011), or it may result simply in a larger body size, which may be maladaptive. These costs may not be worth paying when space is at a premium, or when the energy, materials or information-processing capacity could be put more profitably to other uses. The literature on canalization and plasticity provides well-developed frameworks for analyzing costs (Auld et al., 2010; DeWitt et al., 1998), which could be applied to estimating costs of noise dampening, and I follow this thread no further here.

Noise can be information

Just as one person’s music is another’s noise, extrinsic environmental conditions and sensory inputs can constitute information or noise, depending on whether the input is detectable and relevant to the organism’s performance. In this regard, there is an important distinction between stochastic noise (intrinsic) and incomplete homeostasis (extrinsic). Stochasticity is a kind of white noise, containing no information. Incomplete homeostasis, however, can generate variation (noise) that nevertheless contains information about the environment. For example, in humans, systems devoted to salt and water balance regulate blood osmolality within narrow limits, and we do not have major systems that interpret our surroundings based on the variation within those limits. In this sense, large variation in blood osmolality, were it to occur, would be potentially dangerous noise that would affect the performance of many other systems (Woods and Wilson, 2013). By contrast, many organisms do not regulate their blood osmolality nearly as closely. For example, the blue crab *Callinectes sapidus* inhabits bays and estuaries along the east coast of North America. During their lifetime, individual crabs undergo a complicated set of migrations between full strength seawater and nearly fresh water, which poses significant problems for their osmoregulatory systems (Mangum and Towle, 1977). In these crabs, changes in blood osmolality appear to play a role in coordinating changes in multiple other physiological systems (oxygen transport, pH regulation) so that crab physiology as a whole is retooled to perform well in the various salinities it encounters (Mangum and Towle, 1977).

Noise can enhance performance

Different kinds of noise are known to play constructive roles in organismal development and performance (Losick and Desplan, 2008; MacNeil and Walhout, 2011; McDonnell and Abbott, 2009; Samoilov et al., 2006). One interesting phenomenon is stochastic resonance, in which intermediate levels of background noise allow systems to perform better than systems with either no or lots of noise (McDonnell and Abbott, 2009). In cell biology, known beneficial effects of noise stem from the diversifying effects that noise has on cellular differentiation and function. For example, noise in transcription factors creates the stochastic array of ommatidial sensitivities in *Drosophila* eyes, which collectively make the full range of color vision possible (Wernet et al., 2006), and generate the specific but diverse set of olfactory neurons in mammals (Mombaerts, 1999). Noise in yeast, which generates differential proteins levels among individual cells, can enhance the probability of populations surviving bouts of extreme environmental stress (Blake et al., 2006) or in oscillating environments (Acar et al., 2008). Likewise, noise in bacteria can generate populations of dormant persister phenotypes, which survive environmental insults better than do active cells. This kind of noise-induced variation may represent a kind of bet hedging by bacterial populations (Losick and Desplan, 2008). In other gene circuits, noise can facilitate the emergence of oscillations (Steuer et al., 2003; Vilar et al., 2002), and noise has similar effects in neural networks (Buhmann and Schulten, 1987). Collectively, these benefits seem to derive from the net positive effects of closely associated collections of functional types (Samoilov et al., 2006), an observation which I use below to develop the idea of mosaic physiology.

Mosaic physiology

To summarize the preceding: stochastic developmental noise in multicellular organisms arises from noise in gene networks, and this noise can be transmitted into later life stages by the growth and differentiation of the cell lineages that give rise to later tissues and organs. Cells appear to have sophisticated and evolvable mechanisms for controlling noise (MacNeil and Walhout, 2011); nevertheless, some noise in some gene networks (or among some cells) may be beneficial. Below, I integrate these conclusions into modern ideas about the roles of phenotypic plasticity and flexibility in organisms. From this integration, I derive a new hypothesis about the benefits of developmental noise to the performance of physiological systems in multicellular organisms.

Phenotypic plasticity describes the set of phenotypes produced by a genotype across environments (Pigliucci, 2001). Although it has often been used to denote irreversible phenotypic differences that arise when genotypes are subjected to different environments during development (West-Eberhard, 2003; Wilson and Franklin, 2002), the concept has been broadened in various ways to include a set of short-term, reversible changes in physiology. Piersma and colleagues (Piersma and Drent, 2003; Piersma and van Gils, 2010) have called these sorts of physiological changes ‘phenotypic flexibility’.

There is, however, a serious and misleading consequence of viewing functional diversity in the light of developmental plasticity, a problem which stems from the number of phenotypes per trait that a single organism is assumed to take on. In developmental plasticity, that number is one – i.e. an organism and its genotype interact with some environmental history to produce ‘a phenotype’. The phenotype may differ according to environmental history, in which case it is plastic and displays a reaction norm; or it does not and is said not to show plasticity. In phenotypic flexibility too, the number of phenotypes at a given moment is one, although those phenotypes can change (more rapidly and reversibly) during the lifetime of an organism.

In many physiological systems, however, there is no *a priori* reason to assign one phenotype per genotype at a given moment. This is because physiological systems themselves consist of modules (manifest as collections of tissues, which themselves are collections of cells), and different modules can take on somewhat different phenotypes simultaneously. This observation leads to the new hypothesis: that physiological systems show adaptive mosaic physiology, in which the different modular units, distributed in space inside any organism, exhibit different phenotypes at the same time. The idea of mosaic physiology is also related to ‘homeostatic heterogeneity’, recently proposed by Liao and colleagues (Liao et al., 2012a; Liao et al., 2012b), which characterizes phenotypic differences within populations of human cancer cells. Mosaic physiology can also be thought of as a kind of multicellular version of the phenotypic diversity already known to arise from stochasticity in unicellular populations (Fedoroff and Fontana, 2002; Heinemann and Zenobi, 2011; Raj and van Oudenaarden, 2008).

In multicellular organisms, mosaic physiology can conceivably generate phenotypic diversity having two qualitatively different patterns. The first is analogous to polyphenisms at the level of the whole organism. Polyphenisms describe discrete sets of phenotypes arising from genotypes interacting with environments (Simpson et al., 2011): an organism has phenotype A or B (or C, etc.), they are distinct and there are no intermediates between them. In mosaic physiology, this type of variation describes situations where stochastic noise leads to discrete cell- or tissue-level phenotypes operating in the same physiological system, e.g. the stochastically driven diversity of fly ommatidia described by Wernet and colleagues (Wernet et al., 2006) or populations of different fiber types in vertebrate muscle (Hughes and Salinas, 1999). This pattern could be particularly likely if gene regulatory networks have alternative, discrete stable states, and some form of stochasticity drives initial entry into one of the states. This pattern of variation could be called ‘mosaic polyphenism’. The contrasting pattern of variation, in mosaic physiology, is analogous to continuous forms of plasticity in whole organisms. In this form, genotypes interact with their environments to produce phenotypes, and there is a more-or-less continuous mapping of environments on to phenotypes (for a particular genotype); that map is called a ‘reaction norm’. In mosaic physiology, this kind of variation describes situations in which developmental stochasticity broadens the distributions of cell and tissue functions across some continuous range of possibilities.

What is the function of mosaic physiology? I propose that it builds adaptive diversity into physiological systems – if it occurs at the right levels and in the right systems. A key problem, for any organism, is to modify its set of phenotypes in response to rapidly changing, complex, multivariate environments. The kinds of phenotypic plasticity examined in the past 50 years reflect in large part thinking along two conceptual axes: reversibility and time scale. However, it is likely that organisms are subject to environmental challenges that lie off these axes: (1) novel kinds of multivariate environmental variation that neither they nor their evolutionary lineage have ever experienced; and (2) rapid, multivariate challenges that threaten to overwhelm what would otherwise be well-adapted systems. I propose that mosaic physiology provides a set of standing, diversified phenotypes that provide a greater likelihood of seeing the organism through novel challenges with at least adequate performance (Fig. 3).

It is also important to state what mosaic physiology is not, and to identify constraints on its action. Multicellular organisms having

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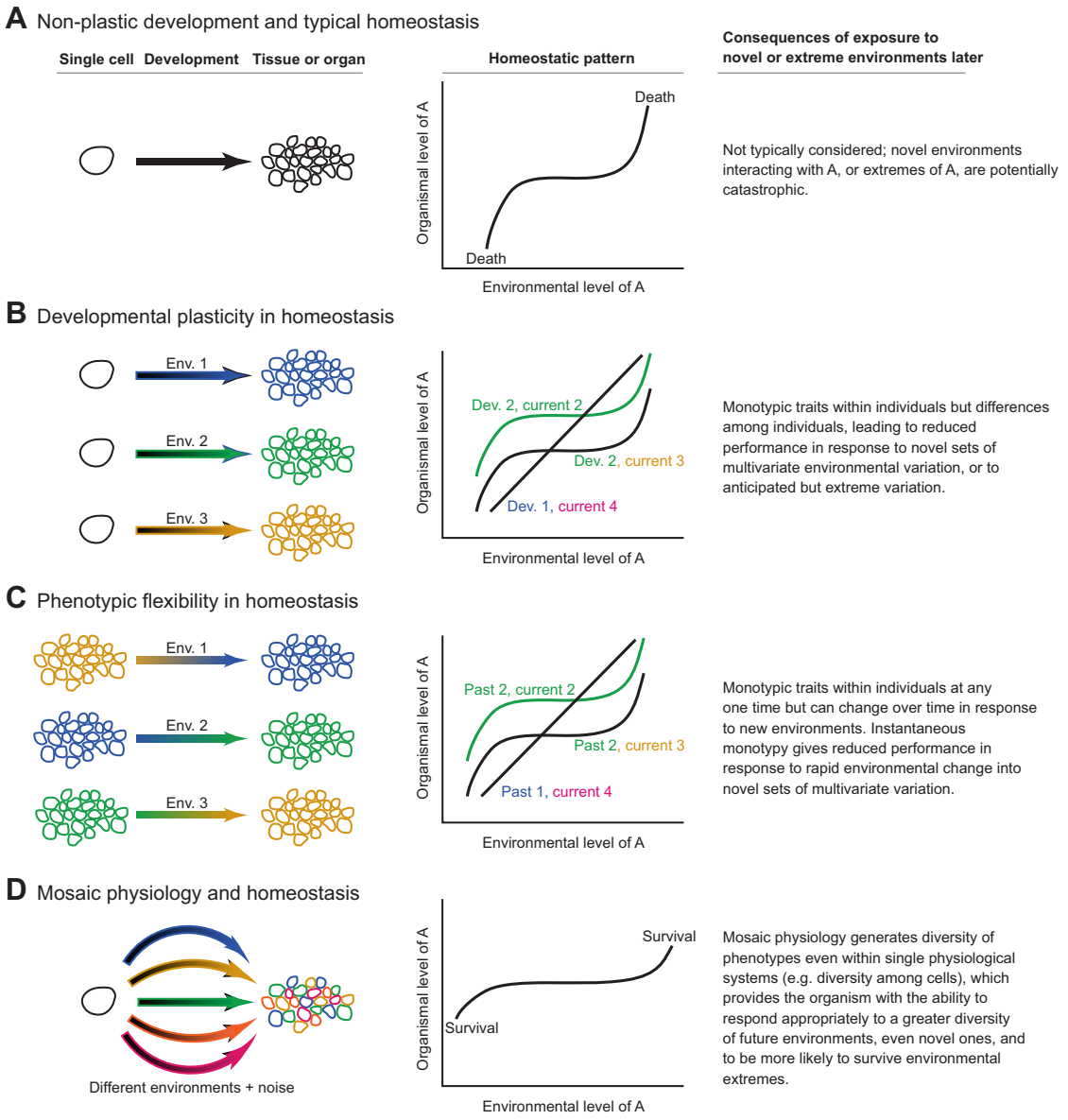

Fig. 3. Homeostasis, phenotypes and mosaic physiology. (A) A typical conception of the development of a homeostatic system. A single cell, or group of cells, gives rise to tissues and organs devoted to controlling some particular factor. The system carries out homeostasis whenever it regulates levels of the factor (here called A) inside the organism, such that internal fluctuations are dampened compared with external or environmental variation in the factor. Often such systems fail at environmental extremes. (B) Developmental plasticity describes particular genotypes giving ranges of phenotypes depending on the environment experienced during development. Developmental plasticity can be adaptive (where the phenotype generated performs better in the current environment than would some other phenotype) or non-adaptive. Importantly, a phenotype, generated by a genotype interacting with an environment, is considered to be monotypic – an individual exhibits 'a phenotype'. If this were the case, we would expect significant mismatches, for some fraction of organisms, between physiological phenotypes produced and environments experienced, such that they had very low fitness. (C) Phenotypic flexibility partially solves the problem of potentially long lag times (between developmental and adult environments) by recognizing that organisms also generate shorter-term, reversible plasticity within their lifetimes. Nevertheless, phenotypic flexibility still views phenotypes as monotypic, which makes organisms vulnerable to novel combinations of environments, or extremely rapid change. (D) Mosaic physiology solves these problems by proposing that individual physiological systems contain multiple phenotypes simultaneously, and that intra-organismal phenotypic diversity is generated in part by stochastic events.

different cell types could be thought of as 'mosaics' – but this description does not capture the essence of mosaic physiology. Whereas the stereotyped differences between one cell type and another often reflect deterministic developmental programs (but see Losick and Desplan, 2008), mosaic physiology focuses on subtler, stochastically driven differences among cells (and tissues and organs) of a particular type. Mosaic physiology is also not conceived as an organismal panacea that could, in excess, solve any functional problem. In this respect, a useful analogy could be drawn between mosaic physiology and specialist–generalist trade-offs in the context of performance curves (Gilchrist, 1995). Specialists have high performance over some narrow range of environments but low performance in others. By contrast, generalists are relatively good across many environments but great in none of them. Neither is necessarily best, and the outcome for any lineage at any point in its evolutionary history will depend on its environments, its genetic variation for performance, etc. In this same way, mosaic physiology could exhibit specialist–generalist trade-offs. A specialist phenotype

would involve very little stochastically driven phenotypic differentiation; such a set of cells may have high performance in well-controlled or anticipated environments. By contrast, a generalist phenotype would perform adequately in many environments, but perhaps not particularly well in any given environment. Here too, the actual outcome in any particular lineage would depend on the relative advantages of specialized *versus* generalized capabilities, the genetic variation available to selection, etc.

How does mosaic physiology arise in organisms? It stems from stochastic developmental noise, which generates differences among modules (tissues, cells) within systems (Fig. 3D). This idea does not supersede developmental plasticity or phenotypic flexibility (Fig. 3B,C), nor does it suggest that noise fundamentally alters developmental programs by causing cells to differentiate into fundamentally different types; rather, it provides an automatic mechanism for broadening their capabilities and for generating mosaics of phenotypic diversity (Acar et al., 2008; Balázsi et al., 2011; Samoilov et al., 2006) that help organisms cope with extreme or unanticipated environments.

The hypothesis of mosaic physiology is a generalization of several key studies emerging from work on mammals, *Drosophila* and *Saccharomyces*. Wernet and colleagues (Wernet et al., 2006) found that stochasticity – in the expression of a single transcription factor (Spineless) in *Drosophila* ommatidia during pupation – generates the retinal mosaic necessary for adult flies to have full color vision. Similarly, stochasticity in the expression of olfactory receptors in mammals generates the full suite of olfactory sensory neurons (Mombaerts, 1999). Using engineered strains of *S. cerevisiae*, Blake and colleagues (Blake et al., 2006) showed that populations in which stochasticity generated variable patterns of gene transcription survived environmental stresses better than did non-noisy populations. Also in yeast, Raser and O'Shea (Raser and O'Shea, 2004) demonstrated noise in the relative expression levels of the two alleles at particular loci, and they suggested that this variability may be beneficial by providing the cell population with a greater range of phenotypes. Mosaic physiology should play analogous roles in physiological systems in all multicellular organisms: cell-level stochasticity should generate diversity in gene expression patterns, giving differences in cell physiological phenotypes, which cascade into different phenotypes among the modules making up any particular system. That diversity then (as in the fly and mammal examples) provides a greater range of functional abilities within any larger unit, and (as in the yeast example) may help organisms perform and survive better during extreme stress.

The concept of mosaic physiology also points to a close functional relationship between extrinsic and intrinsic noise. Extrinsic sources are controlled by negative feedback implemented in homeostatic systems (Cannon, 1932; Woods and Wilson, 2013). Like extrinsic noise, intrinsic noise is dampened by a variety of negative-feedback mechanisms, but in sub-cellular spaces. Mosaic physiology, however, emphasizes that intrinsic noise can be used to construct more effective systems for combatting external noise. The phenotypic diversity present in physiological systems makes them better able to respond appropriately to a variety of external disturbances.

Finally, there is good reason to believe that the degree of mosaic physiology is evolvable – because several known, simple factors affect the stochasticity of expression of messages and proteins. In yeast, for example, Newman and colleagues (Newman et al., 2006) used high-throughput flow cytometry to assess levels of noise in over 2500 different proteins. They found very large differences in levels of noise among proteins, which were associated with the kind of promoter the gene had (which in turn affected how burst-like its transcription was). In addition, different levels of noise were associated with location within the cell. Proteins associated with the Golgi had low variation whereas those associated with mitochondria and peroxisomes had comparatively high variation. Also in yeast, essential genes, compared with randomly chosen genes, were produced by comparatively high rates of transcription and low translational efficiency (Fraser et al., 2004) – which makes them less likely to go through periods of very low copy number. In a third study (Becskei et al., 2005), noise levels were associated with the position of the gene along the chromosome. In multicellular organisms too, there is recent evidence that levels of noise in different loci are under genetic control [in *Arabidopsis* (Jimenez-Gomez et al., 2011)]. Together, this disparate set of results implies that multicellular lineages could evolve optimal levels of noise for particular loci, or particular physiological systems.

Testing the mosaic physiology hypothesis will be non-trivial. So far, the functional diversification of clonal lineages has simply been observed, and, in some unicellular studies, has been related to fitness (Samoilov et al., 2006). To test whether mosaic physiology really occurs in multicellular organisms and, further, whether it can be adaptive, will require a new research program. The first step should be to assess levels of functional diversity among cells within tissues and organs, and whether that diversity affects tissue- and organ-level function. Such an effort will require substantial advances in experimental techniques for measuring organ phenotypes at multiple levels – e.g. among multiple cells and for whole organs – simultaneously. The second step will be to determine whether functional diversity within tissues and organs actually stems from developmental stochasticity or from other, more deterministic developmental events. There are now well-established methods for unicells that allow one to visualize stochasticity in the expression levels of individual messages and proteins, and these are beginning to be applied to metazoans. The next steps will be to apply these techniques to multicellular organisms across multiple developmental stages. In addition, there is the recurring problem of linking up stochasticity in individual molecules to stochasticity in function. Third, we need to be able to experimentally manipulate levels of stochasticity, and thereby degrees of functional diversity, in tissues. Such an approach likely will work best when levels of stochasticity in individual genes can be manipulated, e.g. by modifying their promoters or locations in the genome. Finally, established methods for manipulating stochasticity will allow us to examine links between mosaic physiology and fitness. In particular, levels of mosaic plasticity should be altered and effects on fitness measured, especially across environments having different magnitudes and kinds of variation. In general, the steps outlined above probably will be possible soonest in model metazoans such as flies and nematodes, for which the broadest array of molecular and genetic tools are available.

Conclusions

Biological systems contain two kinds of noise: extrinsic noise driven by fluctuations in the environment that propagate into the organism, and intrinsic noise arising from the finite numbers of entities and interactions inside cells and tissues (Blomberg, 2006). Intrinsic noise is often but not always destructive, and organisms and their cells have evolved sophisticated feedback mechanisms for dampening its effects. However, intrinsic noise also creates biological diversity that acts as a kind of simultaneous, spatially distributed plasticity, which

I propose to call mosaic physiology. Mosaic physiology may play adaptive roles in organisms – because it establishes the cellular foundations for broadening the set of phenotypes expressed by cells, tissues and organs. This kind of variation provides a set of functional phenotypes that may complement other diversity-generating mechanisms like phenotypic plasticity and phenotypic flexibility.

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