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Sloppiness, robustness, and evolvability in systems biology

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The functioning of many biochemical networks is often robust — remarkably stable under changes in external conditions and internal reaction parameters. Much recent work on robustness and evolvability has focused on the structure of neutral spaces, in which system behavior remains invariant to mutations. Recently we have shown that the collective behavior of multiparameter models is most often *sloppy*: insensitive to changes except along a few 'stiff' combinations of parameters, with an enormous sloppy neutral subspace. Robustness is often assumed to be an emergent evolved property, but the sloppiness natural to biochemical networks offers an alternative nonadaptive explanation. Conversely, ideas developed to study evolvability in robust systems can be usefully extended to characterize sloppy systems.

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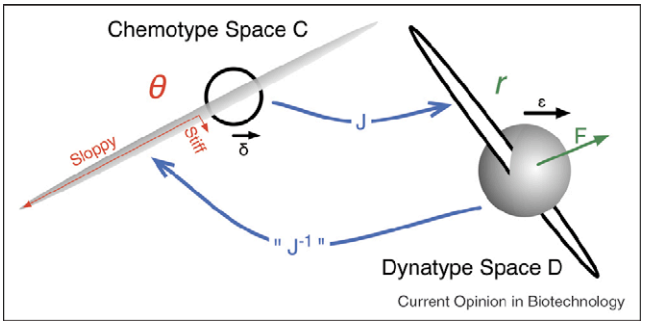
Introduction

Robustness and evolvability are major themes in systems biology, have been the subject of several recent books and reviews [1–5], and have been discussed alongside related phenomena such as canalization, homeostasis, stability, redundancy, and plasticity [6,7,8••,9]. Broadly construed, 'robustness is the persistence of an organismal trait under perturbations' [5], which requires the specification of both traits of interest and perturbations under consideration. Recent work in systems biology has sought to distinguish between environmental robustness (e.g. temperature compensation in circadian rhythms [10,11,12•]) and mutational robustness (e.g. parameter insensitivity in segment polarity patterning [13,14]). Mutational robustness has a subtle relation to evolvability; while allowing survival under genetic alterations, robustness might seem to reduce the capacity for evolutionary adaptation on multigeneration time scales [4,8••].

Earlier robustness work focused on feedback and control mechanisms [15–20]. Much recent work emphasizes neutral spaces and neutral networks: large regions in the space of sequences, parameters, or system topologies that give rise to equivalent (or nearly equivalent) phenotypic behaviors. Neutral spaces have been explored most extensively in the context of RNA secondary structure, where large neutral networks of RNA sequences (genotypes) fold into identical secondary structures (phenotypes) [21–23,8••]. More recently, similar ideas have been applied to neutral spaces underlying the robustness of gene regulatory networks [24,25•,26], where different network topologies (genotypes) can result in identical gene expression patterns (phenotypes). Nontrivial niches in sequence spaces are also seen to emerge in molecular discrimination, a problem where neutral networks allow for biological communication in the presence of uncertainty akin to that found in engineered error-correcting codes [27•]. Functional redundancies and degeneracies arise at many levels of biological organization [28], and it is an important open question as to how neutrality, redundancy, and robustness at different levels are organized and coupled across scales.

Despite these advances in understanding neutral networks connecting genotypes in discrete spaces (e.g. sequences), much of systems biology is focused on chemical kinetic networks that are parameterized by continuous parameter spaces. Often one is interested in the steady-state behavior of a dynamical system, or in the input–output response relating only a subset of the chemical species of a network. In principle, however, one must characterize the full dynamical behavior of a network, in part because any given network may be coupled in unknown ways to other subsystems that are not included in the model. To more clearly delineate distinct levels of biological organization, we have chosen to refer the space of continuous kinetic parameters as a 'chemotype' [29], and to the full dynamical response of a system as its 'dynatype' (Figure 1). The chemotype-to-dynatype maps of interest here are embedded within larger genotype-to-phenotype maps, with chemotypes emerging from lower level processes, and dynatypes contributing to phenotypes and ultimately fitnesses on which selection acts. Recently, there has been an increased interest in characterizing the parametric sensitivity of the dynamics of

Figure 1



Sloppiness in the mapping of chemotypes to dynatypes. It is natural, at least for cellular regulation and metabolic networks, to refine the traditional dichotomy of genotype G to phenotype P by adding two intermediate levels of description, G → C → D → P. Here C is the *chemotype* [29], a continuous description of the behavior in terms of chemical reaction parameters (reaction rates, barriers and prefactors, or Michaelis–Menten parameters). D is the *dynatype*, meant to describe the dynamical responses of the cell (usually the time series of all species in response to selected stimuli, often taken from experimental measurements). Mutations about a particular chemotype $\boldsymbol{\theta}$ occupy a region in chemotype space (here a circle of radius δ), whose image in dynatype space is given by the local Jacobian J of the mapping: mutations along stiff directions in chemotype space will yield large changes in dynatype, while mutations along sloppy directions will lead to small dynamical changes. Conversely, a population of individuals sharing nearly the same dynatype **r** (here a sphere of radius ϵ) will occupy a distorted region in chemotype space, with large variations in reaction parameters possible along sloppy directions (gray ellipse).

biochemical network models, for two important reasons: first, to probe system robustness by quantifying the size and shape of chemotype spaces that leave system behavior unchanged, and second, to characterize system behavior and uncertainties for systems in which precise values for rate constants and other kinetic parameters are typically not known.

Parameter estimation in multiparameter models has long been known to be ill-conditioned: the collective behavior usually cannot be used to infer the underlying constants. Recent work has shown that these models share striking universal features [30,31,32••,33], a phenomenon that we have labeled 'sloppiness' (see Figures 1 and 2). Sloppiness refers to the highly anisotropic structure of parameter space, wherein the behavior of models is highly sensitive to variation along a few 'stiff' directions (combinations of model parameters) and more or less insensitive to variation along a large number of 'sloppy' directions. A nonlinear least-squares cost function can be constructed:

$$C(\boldsymbol{\theta}) = \sum_i \frac{1}{2} \frac{(x(\boldsymbol{\theta}) - x_i)^2}{\sigma_i^2} = \sum_i \frac{1}{2} r_i^2, \qquad (1)$$

where $r_i = (x(\boldsymbol{\theta}) - x_i)/\sigma_i$ is the residual describing the deviation of a dynamical variable x from its measured values x_i with uncertainty σ_i. This cost reflects how well a model with a given set of parameters $\boldsymbol{\theta}$ fits observed experimental data. Parametric sensitivities of the model are encoded in the Jacobian matrix $J = \partial r_i/\partial \theta_j$. The curvature of the cost surface about a best fit set of parameters is described by the Hessian $H_{mn} = \partial^2 C/\partial\theta_m\theta_n$ (or its approximation, the Fisher Information Matrix $J^{\mathrm{T}}J$). Stiff and sloppy directions are conveniently measured using an analysis of eigenvalues λ_n of the Hessian H (Figure 3); large eigenvalues correspond to stiff directions. For a broad range of multiparameter models (e.g. 16 models drawn from the systems biology literature [32••] and models from quantum Monte Carlo, radioactive decay, and polynomial fitting [34•]) these eigenvalues are roughly uniformly spread over many decades, with many sloppy directions a thousand times less well determined than the stiffest, best constrained parameter combinations. Two consequences are that useful model predictions can be made even in the face of huge remaining parameter uncertainty, and conversely that direct measurements of the parameters can be inefficient in making more precise predictions [32••]. Random matrix theory can be used to develop insight into the source of this type of eigenvalue spectrum and the nature of redundancies that appear to underlie sloppiness [34•]. Our open-source code SloppyCell (http://sloppycell.sourceforge.net) provides tools for exploring parameter spaces in systems biology models [35].

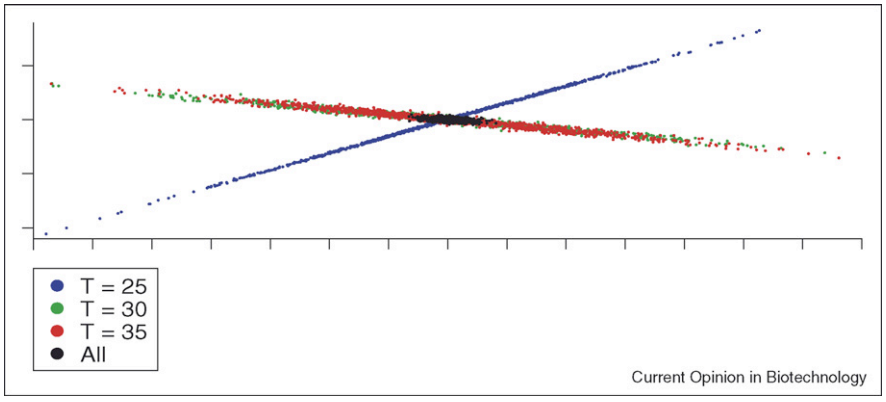
Others have recently addressed similar questions related to the lack of detailed information about kinetic parameters. These include the inference of probabilistic statements about network dynamics from probability distributions on parameter values [36]; the use of 'structural kinetic modeling' to parameterize the Jacobian matrix J and thereby probe ensembles of dynamical behaviors [37,38]; the construction of convex parameter spaces ('k-cones') containing all allowable combinations of kinetic parameters for steady-state flux balance [39]; the use of ideas from control theory, worst-case analysis and hybrid optimization to measure the robustness of networks to simultaneous parameter variation [40]; and exploration of correlated parameter uncertainties obtained via global inversion [41].

Can we connect sloppiness to robustness and evolvability? It is our contention that sloppiness — the highly anisotropic structure of neutral variation in the space of chemotypes — has important implications for how one characterizes robustness in systems biology models. In addition, insights developed in the study of robustness and evolvability suggest new and potentially useful ways of analyzing and interpreting sloppiness.

Environmental robustness and sloppiness

Organisms must thrive under many environmental conditions: changing temperatures, salt concentrations, pH,

Figure 2



Sloppy parameter distributions: dependence on external conditions. Shown is a two-dimensional view of the parameter sets (free energy barriers and prefactors) that accurately predict the experimental phosphorylation dynamics [11] in a 36-parameter subnetwork of a model of circadian rhythms [12•], within a harmonic approximation (see Supplemental Material). Shown are parameters valid at three different temperatures (colors) and valid for all temperatures simultaneously (black). The plot shows one 'stiff' direction in parameter space for each temperature which is tightly constrained by the data, and one 'sloppy' direction which has relatively large variations without change in behavior. Most of the 34 other directions in parameter space not shown are sloppy; the two-dimensional view was chosen to best align with the stiffest direction for each of the four ensembles. The black region describes organisms that are robust to temperature changes in this range. The acceptable region rotates and shifts with temperature, but the sloppiness allows different temperatures to intersect (robust temperature compensation) though all rates are strongly temperature dependent.

nutrient densities, etc. Many organisms have explicit control mechanisms to keep their internal state insensitive to these external changes — these control mechanisms (homeostasis, adaptation, etc.) have been a historical focus in the robustness literature [15,43]. For variations in temperature, however, many organisms do not have such homeostatic control (with the exception of birds, mammals, and some plants) and must instead cope with the exponential Arrhenius temperature dependence of all their reaction rates by some sort of compensatory mechanism [44].

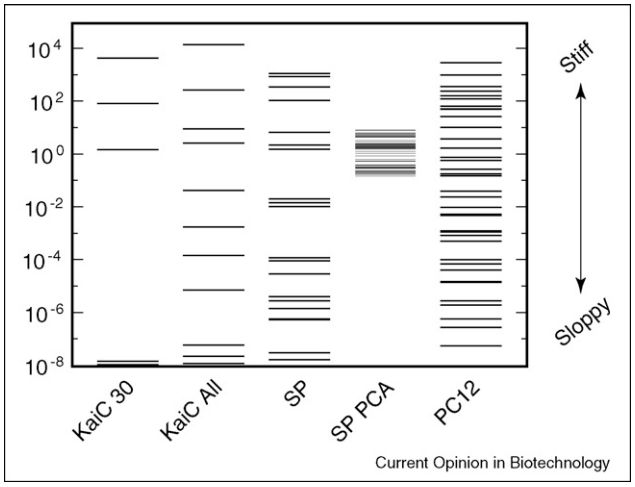
The prototypical example of temperature compensation is the 24-hour period of circadian rhythms [10]. Recent experiments have succeeded in replicating the circadian control network of cyanobacteria in the test tube using three Kai proteins, whose degree of phosphorylation oscillates with a temperature-compensated period in the range of 25–35 °C. In addition, the phosphorylation dynamics of KaiC alone is found to be unchanged as the temperature varies in the same range [11]. This has been cited as a plausible explanation for the observed temperature compensation in the full network, presuming that all other rates are fast [12•] and hence irrelevant to the period. (At least one other explanation of temperature compensation [45] also relies on constraining most rates to be irrelevant). Narrowing our focus to the KaiC phosphorylation subnetwork, however, still leaves the nontrivial task of explaining its temperature compensation mechanism, since estimated energy barriers [46] suggest that phosphorylation rates should be twice as fast at the higher temperature.

The dynamics of KaiC phosphorylation has been modeled using six phosphorylation sites and two conformational states (active and inactive) [12•]. If each of the 18 rates in this model roughly double between 25 and 35 °C, can we adjust the corresponding energy barriers and prefactors such that the resulting net phosphorylation dynamics is temperature independent?

Figure 2 shows a two-dimensional view of the acceptable parameter sets in the resulting 36-dimensional space of energy barriers and prefactors, explored in the harmonic approximation (see Supplemental Material). Notice that the region of acceptable parameters rotates and shifts as the temperature changes. Notice also that the system is sloppy: Figure 2 shows one stiff direction that is highly constrained by the data and one sloppy direction that is largely unconstrained. The eigenvalue analysis in Figure 3 confirms that most directions in parameter space are sloppy and unconstrained. This provides a natural explanation for robustness: the intersection of these large, flat hypersurfaces yields parameters that work at all temperatures.[4] In general, each external condition provides one constraint per stiff direction; since there are only a few stiff directions and many parameters in sloppy models, robust behavior under varying external conditions is easily arranged. Indeed, Figure 3 shows that

[4] In the particular case of KaiC, we find that successful chemotypes favor dephosphorylation in the active state and phosphorylation in the inactive state (see Supplemental Material), so the thermally robust solutions presumably increase the proportion of protein in the inactive state as temperature increases, compensating for the general speedup of all rates.

Figure 3



Sloppy-model eigenvalues. Shown are the eigenvalues of the approximate Hessian J^TJ for the goodness-of-fit $C(\boldsymbol{\theta})$ (Eqn 1) about the best fit. Large eigenvalues correspond to stiff directions; others are sloppy. Notice the enormous range on this logarithmic scale; not all eigenvalues (ranging down to 10^{-20}) are depicted.

- Columns KaiC 30 and KaiC All are for the KaiC phosphorylation dynamics model (Figure 2), showing $T = 30\,^\circ$ C (green region in Figure 2) and simultaneous fits for all temperatures (black region). Notice that the 'robust' simultaneous fit has roughly one more stiff direction than the single temperatures.
- The SP and SP PCA columns are for the segment polarity model [13,42]. SP is an eigenvalue analysis about one of the acceptable parameter sets, showing parameters that keep the behavior (dynatype) of the entire network preserved (time series for all components under all experimental conditions). SP PCA is a principal components analysis of the segment polarity ensemble that yields the wild-type phenotype, with parameters restricted to a relatively small range (roughly three decades each). Most directions in SP are sloppy enough to have fluctuations larger than the sampled phenotype box in SP PCA; the sloppy dynatype SP already explains the robustness to all but a few stiff directions in parameter space. Conversely, the sensitivity of the dynatype SP to a few stiff directions does not preclude phenotypic robustness in those directions for SP PCA; the dynatype (all dynamical evolution) is far more restrictive than the phenotype (output patterning).
- PC12 is for the EGF/NGF growth-factor signaling network [31,32••]; note that it too is sloppy. See Figure 4 for an analysis of evolvability and robustness for this model.

the robust, temperature-independent fits for the KaiC model are themselves a sloppy system.

Chemotype robustness and sloppiness

In addition to robustness to environmental perturbation, biological networks are often robust to mutational perturbations; they maintain their function in the face of mutations that change one or perhaps more of their underlying rate parameters, and thus change their location in chemotype space. Some authors have used this as a criterion for judging model plausibility [47]. The quintessential example of a system that is chemotypically robust is the *Drosophila* segment polarity gene network. Early in development, this network generates a periodic macroscopic phenotype: a pattern of gene expression across several cells that persists throughout development and guides later stages. Multiparameter models of this network [13,14,47,48] find that a surprisingly large fraction of randomly chosen parameter sets generate a pattern consistent with the observed patterning of three genes — the system exhibits chemotype robustness.

In the context of sloppy models, we may define chemotype robustness as the fraction of a given volume in parameter/chemotype space C that maps into a functional region of behavior/dynatype space D (Figure 1). This latter functional region represents behavior close to optimum (or close to that measured experimentally). For simplicity, let us consider it to be a hypersphere of radius ϵ (i.e. a cost $C(\boldsymbol{\theta}) = \sum r_i^2/2 < \epsilon^2/2$ in Eqn 1); larger changes in behavior are considered significantly different, perhaps lowering the organism's fitness. The given volume in chemotype space C might be (as for the segment polarity network) a hypercube of parameter ranges deemed reasonable, or (as a simple model of mutations) a hypersphere; let its scale be given by δ. Our robustness is therefore the fraction of all points in the δ-ball in C that map into the ϵ-ball in D — in Figure 1 the fraction of the circle whose interior is colored gray. This fraction can be calculated (see Supplemental Material) and is approximately given by

$$R_c = \prod_{\lambda_n > \lambda_{crit}} \sqrt{\frac{\lambda_{crit}}{\lambda_n}}, \qquad (2)$$

where $\lambda_{crit} = \epsilon^2/\delta^2$. This formula can be motivated by considering the robust subregion (gray needle intersecting the circle) to be a slab, with thickness $\epsilon\sqrt{\lambda_n}$ along the

eigendirection corresponding to each eigenvalue λ_n.[5] For sloppy directions with $\lambda_n < \epsilon^2/\delta^2 = \lambda_{\text{crit}}$, the slab is thicker than the circle and does not reduce the robust fraction; for each stiff direction with $\lambda_n > \lambda_{\text{crit}}$, the fractional volume is reduced roughly by a factor of the slab thickness $\epsilon\sqrt{\lambda_n}$ over the sphere width δ, leading to Eqn 2.

In their model of segment polarity, von Dassow *et al.* found that approximately 1 in 200 randomly chosen parameter sets generated a wild-type expression pattern for three key genes [13]. This would naively seem amazing for a 48-parameter model like theirs; in an isotropic approximation, each parameter would be allowed only 6% chance of changing the wild-type pattern (since $0.94^{48} \sim 1/200$). However, we have previously shown that the segment polarity model is sloppy [32••]. That is, going far beyond restricting the output phenotype, the dynamical evolution of every component of the network is approximately preserved even with huge changes in parameter values: only a few stiff directions in chemotype space are needed to maintain the dynatype (see column SP in Figure 3). Sloppiness hence provides a natural explanation for the wide variations in all but a few directions in parameter space.

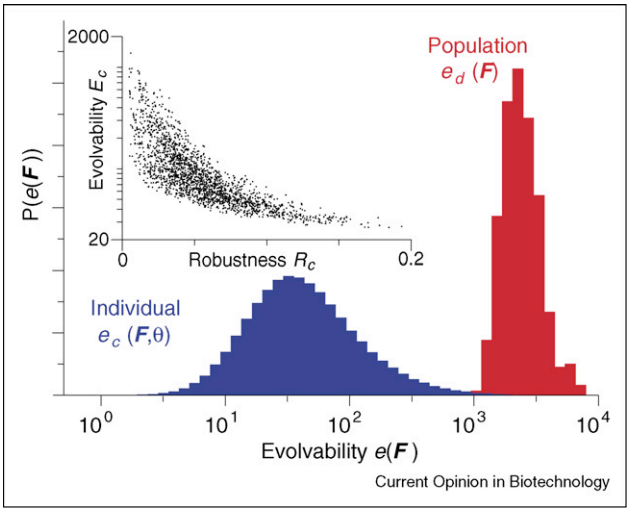
The success rate of 1 in 200 is not nearly as striking if the dynamics is already known to be insensitive to all but perhaps four or five combinations of parameters: $0.35^5 \times 1^{43} \sim 1/200$. Column SP PCA in Figure 3 fleshes this picture out with a principal components analysis (PCA) of the robust region seen in von Dassow *et al.*'s original model, reconstructed using Ingeneue [42]. Note that these PCA eigenvalues are cut off from below by the parameter ranges chosen by the original authors for exploration (typically three decades per parameter). Although the overall scale of the dynatype sloppy-model eigenvalues in SP and the phenotype eigenvalues in SP PCA cannot be directly compared, it is clear that the vast majority of sloppy-model eigenvalues are too small to constrain the parameters within the explored region. The model is robust in these directions not because of evolution and fitness, but because of the mathematical behavior of chemical reaction networks, which are naturally weakly dependent on all but a few combinations of reaction parameters.

Robustness, evolvability, and sloppiness

Mutational robustness of systems would seem to be at odds with an ability to adapt and evolve, since robustness implies persistence of phenotype or function, which may inhibit the capacity for evolutionary change. The concept of neutral spaces has been used — most notably by Wagner and collaborators — to suggest a resolution of this apparent paradox, as demonstrated in model systems exploring various genotype-to-phenotype maps [8••,23,24,25•]. The important insight is that neutral spaces and neutral networks enable systems to drift robustly in genotype space (i.e. without significant phenotypic change), while encountering new and different phenotypes at various points along that neutral space. This insight results from a distinction between the robustness and evolvability of any given genotype, and the robustness and evolvability of all genotypes consistent with a given phenotype [8••].

Evolvability is postulated to reflect the range of possible different phenotypes that are possible under genotypic mutation. How does the sloppy connection between parameters and behavior impinge on the question of evolvability? Translating previous work on discrete genotype and phenotype spaces to the continuous spaces of chemotypes and dynatypes is nontrivial. Since the dimensionality of the space of chemotypes is less than that of dynatypes, the volume of dynatype space accessible

Figure 4



Evolvability and robustness in a sloppy system. Evolvability distributions, and evolvability versus robustness, for an ensemble of parameters for a model of an EGF/NGF signaling pathway fitted to experimental data in PC12 cells [32••]. The histogram on the left is the distribution of individual/chemotype evolvabilities $e_c(\mathbf{F}, \theta_\alpha)$ (Eqn 3), as **F**(an evolutionary pressure in dynatype space) is randomly chosen in direction with uniform magnitude and θ_α varies over the ensemble. The histogram on the right is the corresponding distribution of population/dynatype evolvabilities $e_d(\mathbf{F})$ (Eqn 4). Note that the population evolvabilities are significantly higher than the individual ones. The inset plots the RMS individual chemotype evolvability $E_c(\theta_\alpha)$ versus the robustness $R_c(\theta_\alpha)$ (Eqn 2) for the ensemble. (λ_{crit} is chosen as the fourth-stiffest eigenvalue at the best fit: see Supplemental Material). Note that, for each individual, more robustness leads to less evolvability — individuals which rarely mutate to new forms cannot evolve readily. This need not apply to the population, insofar as we expect robust dynatypes to explore larger regions of parameter/chemotype space, and thus the ratio of dynatype-to-chemotype evolvability to increase with increasing robustness.

[5] The cost for a small displacement of size $\Delta\theta$ along the eigendirection n is $\lambda_n \Delta\theta^2/2$, which equals $\epsilon^2/2$ when $\Delta\theta = \pm\epsilon\sqrt{\lambda_n}$.

under changes in chemotype is zero; the volume in chemotype space maps onto a 'flat' subspace in dynatype space. To develop a sensible definition of evolvability in such systems, we postulate forces $\mathbf{F}$ in dynatype space (Figure 1) that reflect evolutionary pressures due to changes in the environment, such that a change $\mathbf{r}$ in dynatype leads to a change $\mathbf{r} \cdot \mathbf{F}$ in fitness. An organism's evolvability is related to its capacity to respond to external forces through appropriate mutations in chemotype.

For a given force $\mathbf{F}$, the maximum fitness change among mutations of size δ in chemotype space is given by

$$e_c(\mathbf{F}, \boldsymbol{\theta}) = \sqrt{\mathbf{F}^T J J^T \mathbf{F}}\delta \quad (3)$$

which we call the chemotype evolvability distribution (see Supplemental Material). Refs. [31,32••] generate ensembles of parameters (chemotypes) consistent with a given dynatype for an EGF/NGF signaling pathway in PC12 cells, where the dynatype is constrained to fit available experimental data. (The PC12 network is sloppy, see Figure 3.) Each member of such an ensemble $\boldsymbol{\theta}_\alpha$ has a Jacobian J_α. As in Ref. [8••], which distinguishes between genotype and phenotype evolvability, we can distinguish between the chemotype $e_c(\mathbf{F}, \boldsymbol{\theta}_\alpha)$ and dynatype:

$$e_d(\mathbf{F}) = \max_{\theta_\alpha} e_c(\mathbf{F}, \boldsymbol{\theta}_\alpha) \quad (4)$$

evolvability distributions. The first gives the distribution of adaptive responses to $\mathbf{F}$ of individual chemotypes in a population, while the second gives the optimal response within the population. Figure 4 shows the chemotype and dynatype evolvability distributions, generated using the PC12 ensemble of Ref. [32••] and a uniform distribution of force directions $\mathbf{F}$ in dynatype space. Within a population sharing the same behavior, we find substantial variation of accessible behavior changes, leading to a substantially larger population (dynatype) evolvability than individual (chemotype) evolvability. This echoes the finding of Wagner that phenotype evolvability is greater than genotype evolvability for RNA secondary structures [8••].

It is natural to define an overall evolvability as the root-mean-square average of the evolvability distribution over a spherical distribution of environmental forces $\mathbf{F}$ in dynatype space:

$$E_c(\boldsymbol{\theta}_\alpha) = \sqrt{\langle e_c(\mathbf{F}, \boldsymbol{\theta}_\alpha)^2 \rangle_{\mathbf{F}}} \quad (5)$$

and correspondingly for the overall RMS dynatype evolvability. The inset to Figure 4 shows that the chemotype evolvability decreases as the chemotype robustness increases, closely analogous to Wagner's discovery that genotype evolvability decreases as genotype robustness increases, except that his plot averages over phenotypes while ours represents variation within a dynatype. Thus we reproduce Wagner's observation [8••] that individual evolvability decreases with robustness and that population evolvability is significantly larger than individual evolvability.[6]

Conclusion

Our previous work aimed at developing predictive systems biology models in the face of parametric uncertainty has led us to formulate a theory of sloppiness in multiparameter models. The picture that emerges from this theory is of a highly anisotropic neutral space in which variation in parameters (chemotypes) can leave system behavior (dynatypes) unchanged. This picture is reminiscent in many ways to the notion of neutral spaces and neutral networks that has been developed to explore the robustness and evolvability of biological systems. We have been motivated by those ideas to here reconsider sloppiness within that context, both to highlight implications of sloppiness for the study of robustness and evolvability, and to identify new methods for analyzing sloppy systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.copbio.2008.06.008.

References and recommended reading

Papers of particular interest, published within the period of the review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Wagner A: *Robustness and Evolvability in Living Systems*. Princeton University Press; 2005.
2. de Visser JAGM, Hermisson J, Wagner GP, Meyers LA, Bagheri-Chaichian H, Blanchard JL, Chao L, Cheverud JM, Elena SF, Fontana W *et al.*: **Perspective: evolution and detection of genetic robustness**. *Evolution* 2003, **57**: 1959-1972.
3. Jen E (Ed): *Robust Design: A Repertoire of Biological, Ecological, and Engineering Case Studies*. Santa Fe Institute Studies in the Sciences of Complexity, Oxford University Press; 2005.
4. Lenski RE, Barrick JE, Ofria C: **Balancing robustness and evolvability**. *PLoS Biol* 2006, **4**:e428.
5. Felix MA, Wagner A: **Robustness and evolution: concepts, insights and challenges from a developmental model system**. *Heredity* 2008, **100**:132-140.
6. Kitano H: **Towards a theory of biological robustness**. *Mol Syst Biol* 2007, **3**:137.
7. Jen E: **Stable or robust? What's the difference?**. *Complexity* 2003, **8**:12-18.

[6] Unfortunately, we cannot reproduce Wagner's final conclusion (that phenotype evolvability increases with phenotype robustness), since our ensemble (generated to match experimental behavior) is confined to the single PC12 species (dynatype).

8. •• Wagner A: **Robustness and evolvability: a paradox resolved**. *Proc R Soc Lond B Biol Sci* 2008, **275**:91-100.
Introduces the notion of phenotype robustness and phenotype evolvability in which the apparent tension between robustness and evolvability is resolved, and applies these ideas to the study of RNA secondary structures.

9. Krakauer D, Plotkin J: **Principles and parameters of molecular robustness**. In *Robust Design: A Repertoire of Biological, Ecological, and Engineering Case Studies.* Edited by Jen E. Oxford University Press; 2005:71-104.

10. Pittendrigh CS: **On temperature independence in the clock system controlling emergence time in *Drosophila***. *Proc Natl Acad Sci U S A* 1954, **40**:1018-1029.

11. Tomita J, Nakajima M, Kondo T, Iwasaki H: **No transcription-translation feedback in circadian rhythm of KaiC phosphorylation**. *Science* 2005, **307**:251-254.

12. • van Zon JS, Lubensky DK, Altena PRH, ten Wolde PR: **An allosteric model of circadian KaiC phosphorylation**. *Proc Natl Acad Sci U S A* 2007, **104**:7420-7425.
Presents a model of circadian rhythms in cyanobacteria, from which we extracted the submodel for KaiC phosphorylation dynamics.

13. von Dassow G, Meir E, Munro EM, Odell GM: **The segment polarity network is a robust developmental module**. *Nature* 2000, **406**:188-192.

14. Chaves M, Sontag ED, Sengupta AM: **Shape, size and robustness: feasible regions in the parameter space of biochemical networks** 2007. URL: http://arXiv.org/abs/0710.4269.

15. Barkai N, Leibler S: **Robustness in simple biochemical networks**. *Nature* 1997, **387**:913-917.

16. Alon U, Surette MG, Barkai N, Leibler S: **Robustness in bacterial chemotaxis**. *Nature* 1999, **397**:168-171.

17. Yi TM, Huang Y, Simon MI, Doyle J: **Robust perfect adaptation in bacterial chemotaxis through integral feedback control**. *Proc Natl Acad Sci U S A* 2000, **97**:4649-4653.

18. Doyle J, Csete M: **Motifs, control, and stability**. *PLoS Biol* 2005, **3**:e392.

19. Goulian M: **Robust control in bacterial regulatory circuits**. *Curr Opin Microbiol* 2004, **7**:198-202.

20. Kurata H, El-Samad H, Iwasaki R, Ohtake H, Doyle JC, Grigorova I, Gross CA, Khammash M: **Module-based analysis of robustness tradeoffs in the heat shock response system**. *PLoS Comput Biol* 2006, **2**:e59.

21. Schuster P, Fontana W: **Chance and necessity in evolution: lessons from RNA**. *Physica D* 1999, **133**:427-452.

22. Fontana W: **Modelling 'evo-devo' with RNA**. *Bioessays* 2002, **24**:1164-1177.

23. Sumedha, Martin OC, Wagner A: **New structural variation in evolutionary searches of RNA neutral networks**. *Biosystems* 2007, **90**:475-485.

24. Ciliberti S, Martin OC, Wagner A: **Robustness can evolve gradually in complex regulatory gene networks with varying topology**. *PLoS Comput Biol* 2007, **3**:e15.

25. • Ciliberti S, Martin OC, Wagner A: **Innovation and robustness in complex regulatory gene networks**. *Proc Natl Acad Sci U S A* 2007, **104**:13591-13596.
Examines the structure of neutral networks and connections between robustness and evolvability in a simple model of gene regulatory networks.

26. Bergman A, Siegal ML: **Evolutionary capacitance as a general feature of complex gene networks**. *Nature* 2003, **424**:549-552.

27. • Myers CR: **Satisfiability, sequence niches, and molecular codes in cellular signaling**. *IET Systems Biology* 2008, in press. URL: http://arXiv.org/abs/q-bio/0702042.
Explores the nontrivial structure of neutral spaces that arise from the satisfaction of conflicting constraints in a genotype-to-phenotype map relating protein sequences to the avoidance of crosstalk.

28. Edelman GM, Gally JA: **Degeneracy and complexity in biological systems**. *Proc Natl Acad Sci U S A* 2001, **98**:13763-13768.

29. Gutenkunst RN, Sethna JP: **Adaptive mutation in a geometrical model of chemotype evolution** 2007. URL: http://arXiv.org/abs/0712.3240.

30. Brown KS, Sethna JP: **Statistical mechanical approaches to models with many poorly known parameters**. *Phys Rev E* 2003, **68**:021904.

31. Brown KS, Hill CC, Calero GA, Myers CR, Lee KH, Sethna JP, Cerione RA: **The statistical mechanics of complex signaling networks: nerve growth factor signaling**. *Phys Biol* 2004, **1**:184.

32. •• Gutenkunst RN, Waterfall JJ, Casey FP, Brown KS, Myers CR, Sethna JP: **Universally sloppy parameter sensitivities in systems biology models**. *PLoS Comput Biol* 2007, **3**:e189.
Demonstrates the universality of sloppy eigenvalue spectra in chemical kinetic network models from systems biology, and highlights the inefficiency of constraining models by direct parameter measurement because of this sloppy structure.

33. Gutenkunst RN, Casey FP, Waterfall JJ, Myers CR, Sethna JP: **Extracting falsifiable predictions from sloppy models**. *Ann N Y Acad Sci* 2007, **1115**:203-211.

34. • Waterfall JJ, Casey FP, Gutenkunst RN, Brown KS, Myers CR, Brouwer PW, Elser V, Sethna JP: **Sloppy-model universality class and the Vandermonde matrix**. *Phys Rev Lett* 2006, **97**:150601.
Discusses the mathematical origins of sloppy behavior in multiparameter models, with examples of sloppy model behavior outside of biology.

35. Myers CR, Gutenkunst RN, Sethna JP: **Python unleashed on systems biology**. *Comput Sci Eng* 2007, **9**:34-37.

36. Liebermeister W, Klipp E: **Biochemical networks with uncertain parameters**. *IEE Proc Syst Biol* 2005, **152**:97-107.

37. Steuer R, Gross T, Selbig J, Blasius B: **Structural kinetic modeling of metabolic networks**. *Proc Natl Acad Sci U S A* 2006, **103**:11868-11873.

38. Grimbs S, Selbig J, Bulik S, Holzhutter HG, Steuer R: **The stability and robustness of metabolic states: identifying stabilizing sites in metabolic networks**. *Mol Syst Biol* 2007, **3**:146.

39. Famili I, Mahadevan R, Palsson BO: **k-cone analysis: determining all candidate values for kinetic parameters on a network scale**. *Biophys J* 2005, **88**:1616-1625.

40. Kim J, Bates DG, Postlethwaite I, Ma L, Iglesias PA: **Robustness analysis of biochemical network models**. *IEE Proc Syst Biol* 2006, **153**:96-104.

41. Piazza M, Feng XJ, Rabinowitz JD, Rabitz H: **Diverse metabolic model parameters generate similar methionine cycle dynamics**. *J Theor Biol* 2008, **251**:628-639.

42. Meir E, Munro EM, Odell GM, Dassow GV: **Ingeneue: a versatile tool for reconstituting genetic networks, with examples from the segment polarity network**. *J Exp Zool* 2002, **294**:216-251.

43. Stelling J, Sauer U, Szallasi Z, Doyle FJ, Doyle J: **Robustness of cellular functions**. *Cell* 2004, **118**:675-685.

44. Ruoff P, Rensing L, Kommedal R, Mohsenzadeh S: **Modeling temperature compensation in chemical and biological oscillators**. *Chronobiol Int* 1997, **14**:499-510.

45. Hong CI, Conrad ED, Tyson JJ: **A proposal for robust temperature compensation of circadian rhythms**. *Proc Natl Acad Sci U S A* 2007, **104**:1195-1200.

46. Cheng Y, Zhang Y, McCammon JA: **How does activation loop phosphorylation modulate catalytic activity in the cAMP-dependent protein kinase: a theoretical study**. *Protein Sci* 2006, **15**:672-683.

47. Ma W, Lai L, Ouyang Q, Tang C: **Robustness and modular design of the Drosophila segment polarity network**. *Mol Syst Biol* 2006, **2**:70.

48. Ingolia NT: **Topology and robustness in the Drosophila segment polarity network**. *PLoS Biol* 2004, **2**:e123.