Robustness in Biological Systems - A Provisional Taxonomy

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Abstract

Biology is a domain of tension: on the one hand biology is concerned with transformation and the generation of diversity; on the other hand, biology is concerned with the persistence of improbable structural regularitites. Robustness, as a research program, aims to uncover those evolved mechanisms promoting the persistence of regularities. Here I organize mechanisms of robustness into a phenomenological taxonomy, grouping biological mechanisms into principles of robust organization. These include: Redundancy, Purging, Feedback, Modularity, Spatial Compartmentalization, Distributed Processing and the Extended Phenotype. I review studies in which mechanisms representative of each principle are described.

1 A fundamental biological dichotomy: Robustness and Evolveability

Biologists have been motivated by two fundamental sets of questions. One set is associated with the generation and maintenance of genotypic, phenotypic and functional diversity. The second set is associated with genotypic, phenotypic, and functional invariance. Evolutionary theory, following Darwin (1859, 1874), has been concerned largely with transformation – from one species into another with coincident suites of modified adaptive complexes. Mechanistic biology – to include physiology and cell biology – has focused on mechanisms underlying robustness of the genotype and phenotype. Thus not only are robustness and evolvability obverse trends in biological system mechanics, they are also represented by two largely independent research traditions: the historical sciences relying on comparative data and theory, and the ahistorical sciences relying on laboratory data and description.

This caricature of our predicament suggests that two quite separate problems need to be overcome in order to develop unified theories of biosystems. One is to establish the utility of evolutionary thinking in mechanistic science, and the other is to impress the importance of robustness upon evolutionary theory. Such a project would go some way towards reintroducing the phenotype into evolutionary theory.

Much has been written on the subject of transformation. Population genetics is concerned with the study of changing gene frequencies through time (Kimura 1985). Quantitative genetics is concerned with the change in the mean and variance of phenotypes across generations (Falconer 1996). In neither case has it been possible to explicitly incorporate detailed mechanistic components of the phenotye into these models. A recent movement in this direction involves work on the *genotype to phenotype map* and the *representation problem* (Wagner and Altenberg 1994). The genotype phenotype map describes the process of development required to decode a genome into a viable phenotype. The representation problem is concerned with the way in which the variational properties of the genome are dependent upon the precise manner in which phenotypes are encoded in genotypes. To put it another way, are all phenotypes equally accessible from a given genotype configuration, and if not, does this depend upon the way in which phenotypes are represented in genetic data structures? Assuming a fixed representation, are there some phenotypes that are unlikely to ever be realized even in the face of overwhelming selective advantage? If this is so, then these impediments to isotropic adaptive transformation, are likely to be associated with just those mechanism ensuring the unity of type, the stability of genomes across generations, and the homeostatic stability of the phenotype.

One path through the labyrinth of biological robustness is to keep hold of two Ariadne's threads: one connected to limits to evolvability and associated mechanisms limiting variation, and the other, connected to mechanistic inquiries into homeostasis and the regulation of cellular and individual phenotype (Gould 2002).

2 Genotypic versus Environmental versus Functional robustness

When speaking of robustness it is worth bearing in mind the plethora of definitions the word attracts. For an extensive list see [www.discusss.santa.edu/robustness]. These are to some degree domain-specific. In ecology stability or robustness is a measure of the preservation of species diversity upon species removal (May 1973) or, the permanence of a configuration when perturbing some variable of ecological interest. In medicine, robustness is associated with healing and compensation, neither of which imply a return to the original phenotype but rather a restoration of wildtype function (Stearns 1998). In linguistics robustness relates to competence and comprehensibility despite incomplete information and ambiguity (Nowak and Krakauer 1999). Thus structural transformation is acceptable subject to information remaining decodable. In paleontology robustness relates to the continuity of lineages across geological eras

(Erwin 2001), and the persistence of lineages during mass extinction events. In metabolism robustness relates to limited phenotypic variation across large changes in kinetic parameters (Westerhoff, 1984, Hurst 2000). In cell biology robustness can describe how cell fate decisions remain constant when transcription regulation is stochastic (Kepler 2001), or how conserved RNA secondary structures can remain resistant to point mutations (Fontana 2002).

In each of these cases robustness relates to either: (1) non-detectable or minor modification in phenotype following a large perturbation to the genotype, (2) non-detectable or minor modification in phenotype following a large perturbation to the phenotype from the environment (3) non-detectable or minor modification in function following a large perturbation to the genotype or phenotype with or without a correlated change in the phenotype. The important distinction between genotypic and environmental robustness is that in the first case perturbations are inherited, whereas in the second case, they are not. Functional robustness can be achieved through phenotypic invariance or phenotypic plasticity. In one case the phenotype resists perturbations, and in the second case, the phenotype tracks perturbations. Genotypic and environmental robustness can be measured through the environmental (V_e) or mutational variance (V_m) of a trait, where as functional robustness can be measured as the variance in geometric mean fitness. It is often the case that a single mechanism leads to all three forms of robustness in which case we observe *congruence* (Ancel and Fontana 2000) between the genotype and phenotype.

3 Principles and parameters of Robust Organization

In Krakauer and Plotkin (2003), we describe three *principles* that have arisen in the effort to understand the evolutionary response to mutations. The principle of canalization, the principle of neutrality and the principle of redundancy. We contrast these with the *parameters* of robustness – those mechanisms by which these principles are realized. The principles and parameters metaphor is derived from linguistics (Chomsky 1981) where the principles are the invariant properties of universal grammar and the parameters the local rules and practices of language. Here we extend these principles to include: feedback, modularity, spatial compartmentalization, distributed processing and the extended phenotype. Another way of thinking about the principles are as higher grades in a theoretical taxonomy of robustness. All mechanisms employing some form of redundancy are classed together, as are those employing modularity and so on. As we work down the classificatory tree of robustness, we eventually hit the unique mechanical instanstiation giving rise to robustness. Our classification is more Linnean than Darwinian, as we have no external principle with which to organize mechanism.

We give a brief introduction to each of these principles below, and subsequently go on to discuss in more detail, a few models developed to address specific robustness mechanisms in biology.

3.1 Redundancy

A common means of identifying the function of a gene is to perform a knockout experiment, removing or silencing a gene early in development. By assaying the resultant phenotype, the putative function of the absent gene can be inferred. In many such experiments, there is no scoreable phenotype: the knockout leaves the phenotype in the wildtype condition. Biologists refer to a gene x on a background y as functionally redundant (Tautz 1992). This is taken to mean that the target gene is one of at least two or more genes contributing to the phenotype epistatically (Krakauer and Nowak 1999). Removal of a redundant gene x leads to compensation by remaining members of a redundant set $\mathbf{y}$. Let $f(g)$ be the fitness of gene or genome g, then redundancy implies that $f(x, \mathbf{y}) = f(\mathbf{y})$. When $\mathbf{y}$ has a cardinality of one and $\mathbf{y} = x$, then functional redundancy reduces to the special case of a redundant copy of x. Redundancy as a principle, is more general, and describes any case in which the mechanism of robustness is only operative upon petubation. Hence redundancy is a variational property, not contributing to fitness directly, but indirectly operating at the population level. Individuals with a redun-

dancy property are not fitter than those without, but those without, will on occasion suffer the consequences.

True redundancy might be rarer than "artefactual" redundancy, or experimental neutrality, in which the effect of perturbation remains below an experimental detection limit (Ponte at al 1998). Assuming that we are able to detect small changes, the degree of redundancy describes the degree of correlation among genes contributing to a single function. Models of redundancy in biology tend to focus on the evolutionary preservation of redundant components - and hence employ population genetics approaches. More recently differential equation based models for the dynamics of regulatory systems following structural perturbation have also been explored (Wagner 1996).

3.2 Feedback control

Elementary feedback control systems have three components: *a plant* (the system under control), *a sensor* (measuring the output of the plant) and *a controller* (generating the plants input) (Emanuel 1979). A measure of performance is often the degree to which the output of a plant approximates some function of the input to the controller. In biology a plant could be, RNA or protein concentration, protein kinase activation, immune effector cell abundance, or species abundance. Inputs in each of these cases would be transcription factors, protease concentrations, chemical agonists bound to receptors, antigen concentrations and death rates. The controllers are more often than not aggregates of several mechanisms. Feedback is a mechanism of robustness as it enables plants to operate efficiently over a range of exogenous input values. The question remains as to whether the controller is robust to variations in the plant - does it provide *robust stability*? For example, in biology, can a single feedback controller regulate the concentrations of several different proteins?

The theoretical literature in linear feedback control is very well developed in engineering. Biology has borrowed extensively from this literature. Non-linear feedback control is another issue, and there are few canonical models (Aeyels et al 1999).

3.3 Modularity

Independent representations of functionally distinct character complexes capable of recombination or shuffling is an example of modularity. In genetics, modularity involves a minimum of pleiotropy, in which sets of genes contributing to one complex or trait (for example organ system), make little contribution to other complexes or traits (Goldberg 1995, Raff 2000). These modular genetic systems are found in different genomic contexts performing a similar function. Of course modularity can be defined at levels of organization above that of the gene (Winther 2001) - the extent to which organs operate independently during homeostasis. The dissociability of modules provides one means of damage limitation through encapsulation.

There are no collectively agreed upon models for analysing modularity in biosystems. To date quantitative genetics models have been used to explore the limits to the evolution of modularity and neural network models have been used to explore how modularity can lead to more efficient task management (Calabretta et al 1998).

3.4 Purging – antiredundancy

Whereas redundancy buffers the effect of perturbation, purging acts in the opposite fashion –amplifying the effects of perturbation – so as to ensure the purity of a population (Krakauer and Plotkin 2002, Krakauer and Sasaki 2002). A gene x on a genetic background $\mathbf{y} + \mathbf{z}$ is functionally anti-redundant when the target gene

is

one of at least two or more genes $(x + \mathbf{y})$ contributing to the phenotype epistatically, and, when removal of gene x leads to a greater perturbation in the presense of $\mathbf{y}$ than in the absence of $\mathbf{y}$: $f(x, \mathbf{y} + \mathbf{z}) \geq f(\mathbf{z}) >> f(\mathbf{y} + \mathbf{z})$.

Purging is only effective when individual replication rates are sufficiently large to tolerate the effects of removal of defective components. Thus apoptosis - programed cell death - is a common strategy for eliminating cells upon damage to their genomes or upon infection, provided these cell types are capable of regeneration. Nerve cells and germ cells produce factors that strongly inhibit apoptosis (Matsumototo 1999), as removal in these cases, has deleterious consequences. In case of severe infection it can make sense ot purge nerve cells (Krakauer 2000).

Recent models dealing with purging-type phenomena have involved stochastic models assuming finite populations.

3.5 Spatial compartmentalization

Compartmental systems are those made up from a finite number of macroscopic subsystems called compartments, each of which is well mixed. Compartments interact through the exchange of material (Jacquez 1985). The spatial compartmentalization of reactions leads to robustness by minimizing covariance among reaction components participating in functionally unrelated processes. Thus spatial de-correlation through compartmentalization substitutes for temporal correlation in biological functions. Robustness is achieved in at least two ways: (1) minimizing interference - chemical, epistatic or physiological, and (2) minimizing mutual dependencies and thereby attenuating the propagation of error through a system. The study of spatial compartmentalization is particularly rich in theoretical ecology and epidemiology (Levin 1997) where it has been used to explore the maintenance of antigenic diversity, restrictions on pathogen virulence, and seasonal forcing.

From a modeling perspective, compartmentalization is often approached from the perspective of metapopulation dynamics or coupled oscillators, in which space is assumed to be discrete (implicit space) and non-local (Hanski 2001). An alternative approach is based on continuous space (explicit space) with local interactions and employs partial differential equations to study diffusion and advection of components (Murray et al 1996). A third approach assumes discrete space with local interactions employing coupled map lattices and cellular automata. A fourth approach analyzes the statistical connectivity properties of undirected graphs and their response to node or edge elimination (Albert et al 2000).

3.6 Distributed Processing

Distributed processing describes those cases in which an integrated set of functions are carried out by multiple, semi-autonomous units (McClelland 1988, Hertz et al 1991). The most obvious example is that of nerve cells comprising the nervous system. Distributed processing ,or connectionism, might be assumed to be a combination of modularity and spatial compartmentalization, but differs in that a single function is emergent from the collective activities of units, and correlated activity, is thereby a desired outcome.

The robustness properties of connectionist models are: (1) the ability to identify incomplete patterns, (2) generalize from a subset of learnt patterns, and (3) degrade gracefully upon remove of individual nodes.

Connectionist models range from a simple application of linear algebra, dynamical systems and Hamiltonian representations of steady states, through to the use of statistical mechanics models of frustrated systems such as spin glasses.

3.7 Extended Phenotypes

The extended phenotype concept was introduced by Dawkin's (1982) as a means of emancipating the gene from the discrete vehicle (often taken to be the individual organism). Thus while the gene's most proximal effect is to encode proteins, more distally, and as a byproduct, these participate in cells, tissues, organs, individuals, behaviors, mental states and on through to cultures. There is no implication of determinism or strong causality in this statement. The

extended phenotype notion merely recognizes that the boundary of physical embodiment need not represent the boundary of genic action.

In non-human biosystems the importance of the extended phenotype to robustness is not contested – from animal artifacts: ant nests, termite mounds, bird nests, and spider webs – and from animal behavior: policing, reconciliation and dominance. In human society the issue is more controversial and the evidence correspondingly weaker. However it remains a fascinating question to pose - to what extent do human institutions represent instances of mechanisms for biological robustness ? In the non-reductive (gene-independent) example of medical care and hospitals the case is obvious. There are however indications that behavioral rules, such as reciprocity and sharing, are to some extent causally related to the actions of our genes (Constantino and Todd 2000).

Modeling in this area tends to be either game theoretical (Maynard Smith1982) or some variant of population genetics to allow for both vertical and horizontal transmission. This is a nascent field for theory.

4 Case studies of robust principles

In the remainder of this chapter I have chosen case studies to illustrate the application of theory in the study of biological robustness. I have done so, because as of yet there is no unified theory of biological robustness, only collections of illustrative models. These models vary in the degree to which they deal with robustness explicitly, and yet all them bear on the question in some fundamental way.

4.1 Redundancy in genetic networks

Wagner (1994) has studied dynamical models for the evolution transcription regulation circuits. Gene duplication is thought of as a mutational event necessary to establish the genetic diversity for subsequent diversity in spatio-temporal patterning during development. Wagner poses the question: what is the average proportion of genes likely to be involved in a duplication event, such that the initial effect on the phenotype of duplication, is minimized? In other words, what fraction of genes is capable of performing redundantly? This questions can be inverted by asking how many genes from a portion of genome made up from duplicate sets, can be deleted and made to preserve the same phenotype? In the first case, the perturbation involves adding genes and in the second, eliminating genes. Wagner models the gene expression dynamics in much the same way connectionist modelers describe neural networks. The activity of a gene i is denoted by S_i. The magnitude of transcriptional activation between gene i and gene j is given by weight matrix entry w_{ij}. The dynamics of gene expression in discrete time are,

$$S_i(t+\tau) = \sigma[\sum_{i}^{N} w_{ij} S_j(t)] = \sigma[h_i(t)]$$

The function $\sigma[.]$ is the sign function. The output of interest is the steady state levels of gene expression in the network $\bar{\mathbf{S}}$ as a function of the initial conditions of gene expression $\bar{\mathbf{S}}(0)$ and the network connectivity. Whereas duplication (duplication function π) of one or more genes (k) creates a network in a higher dimensional state space, deletion (deletion function δ) creates a network in a lower dimensional state space:

$$\pi : \{-1,1\}^N \rightarrow \{-1,1\}^{N+k}$$

and hence

$$(S_1, \ldots, S_k, S_{k+1}, \ldots, S_N) \rightarrow (S_1, S_1, \ldots, S_k, S_k, S_{k+1}, \ldots, S_N)$$

and for deletions

$$\delta : \{-1,1\}^{N+k} \rightarrow \{-1,1\}^N$$

and hence

$$(S_1, S_1, \ldots, S_k, S_k, S_{k+1}, \ldots, S_N) \rightarrow (S_1, \ldots, S_k, S_{k+1}, \ldots, S_N)$$

Wagner compares the wildtype equilibrium states ($\bar{\mathbf{S}}$) and the state following duplication ($\pi : \bar{\mathbf{S}}$) using Hamming distance between ($\bar{\mathbf{S}}$) and ($\pi : \bar{\mathbf{S}}$) as the robustness metric. It is observed that small duplications and large duplications have the least impact on phenotypic change. And hence small and large deletions are likely to have the least impact on phenotype. Intermediate sized duplications (around 40% of genes) have the greatest impact on phenotype. In a region of the genome made up from sets of duplicate genes, perturbations involving deletions of just under half of the genome, are expeted to have the greatest effect on the phenotype, whereas genotypes are expected to be robust against perturbations involving a few or almost all genes.

Redundancy in this model does not refer to the duplicate genes, but the phenotypic invariance relating to epistasis in the transcriptional network. The explanation for this result is fairly obvious. Duplicating all the genes leaves the network effectively unchanged. Dynamics are not influenced only numbers. Small numbers of duplications proportionately influence a small number of connected pairs. Intermediate sized duplications are likely to be most disruptive.

4.2 Modularity in genetic regulatory networks

In Drosophila the anterior-posterior body axis is segmented. Segmentation is initiated by maternal factors at the embryo stage. Those factors initiating segmentation are expressed transiently, and it is left to a segment polarity network to maintain the definition of segment boundaries. Segment polarity networks abound in insect orders, whereas the patterns of stable segmentation, are variable. Von Dassow et al (2000, 2002) suggest that the segment polarity network is a robust evolutionary module, recruited by different insect species, and provided with different inputs to produce diverse patterns of segmentation. In order for this to be the case, parametric variation in reaction coefficients, should leave the patterning ability of the network in tact.

In order to model the network, Von Dassow simulate large systems of coupled first order differential equations. For example, the rate of transcription of mRNA M_i from gene E_i assuming a concentration of binding transcription factor x_i, a maximum rate of transcription T_{max}, and a rate of decay de_i is given by,

$$\dot{m_i} = T_m a x[\frac{x_i^c}{k^c + x^c}] - dm_i$$

where the parameter k determines the value at which the transcription factor X_i has half maximum effect on the rate of translation of the gene E_i. The subsequent translation of M_i into a protein P_i with a maximum rate of translations r_{max} and a rate of decay $d_p p_i$ is of the form,

$$\dot{p_i} = r_{max}[\frac{m_i}{K}] - d_p p_i$$

These proteins are then free to bind to other proteins forming complexes with novel transcription activity (e.g. a p_i might bind to a p_j to induce x_k etc).

Equations of this form assume saturation of enzymes and substrates. As a consequence, over large variations in parameter values, steady state concentrations of protein products and complexes remain unchanged. Saturation is the assumption behind the derivation of the familiar Michelis Menten rate law: the concentration of substrate is in large excess over the concentration of enzyme (Jordan 1979). In the limiting case of very high values of the constant c, coupled differential equations can be effectively replaced by boolean networks. In this case, only the topology of the network and the initial conditions, not the kinetic constants have an influence on steady states. Thus stable variation of segmentation in insect orders might be achieved through variation in initial conditions with disregard for variation in kinetic parameters. Species diversity would derive from feeding different initial conditions through the same network without regard for species-specific variation in rate constants. If saturation is not justified this robust modularity disappears. The empirical validity of saturation in developmental networks remains to be established.

4.3 Feedback control in immune regulation and signal transduction

4.3.1 Segel and Bar-Or's adaptive control model for immune effector action

The immune system is configured so as to maximize damage to pathogens and minimixe damage to *self*. These however are not orthogonal goals, and hence the regulation of infection by the immune system requires feedback control, in order to prevent an overenthusiastic immune response from destroying healthy tissues.

Segel and Bar-Or (1999) approach the problem as follows. Assume a population of immune effector cells E, a population of pathogens P and a noxious chemical N. The E are able to kill P as is N. However N can also damage the host and thereby compromise the production of E. It is assumed that the immune system seeks to minimize damage to the host by maximizing the efficiency of the immune response. Damage to the host δ is calculated as the time averaged abundance of P and N where damage from P occurs at a rate h_pP and damage from N at a rate h_NN. Thus

$$\delta = \frac{1}{T}\int_0^T [h_pP(t) + h_NN(t)]dt$$

Assuming the dynamical system:

$$\dot{N} = sE - g_NN$$

$$\dot{P} = rP - aEPN$$

$$\dot{E} = E[\mu_pP(1 - E/E_{max}) - g_E]$$

where the crucial parameter s the secretion coefficient of noxious chemicals, in response to immune activation, is assumed to be under constitutive control by the host. The function $\delta(s)$ has a unique minimum for any given value of the pathogen proliferation coefficient r, moreover $d(\delta(s))/dr > 0$.

The problem for feedback control is to determine the optimal value of s for a variety of pathogens with different proliferation rates. Segel and Bar-Or suggest one way, which requires that the host employs two performance measures: a *kill indicator* a chemical K produced in response to immune activity NPE, and a *harm indicator* a chemical produced in response to instantaneous damage – $h_pP + h_NN$. Include these two chemicals in the dynamical system:

$$\dot{K} = c_k(aEPN) - g_kK$$

$$\dot{H} = c_h(h_pP + h_NN) - g_hH$$

Now harm from the pathogen (H_p) is not the same as harm inflicted indirectly through the immune reponse (H_I). Estimate $H_p = H/(1 + k_pN)$ and assuming that $H = H_I + H_p$, then an adaptive s coefficient might change according to the Michaelean rate law:

$$s = s_1 + \frac{s_2KH_p}{1 + s_3H_I + s_4KH_p}.$$

An immune response making use of multiple sources of feedback information can operate effectively over a far greater range of parameter values and variable values than one without. This form of Robustness through feedback control is typical of biological systems.

One caveat to be observed at this point regards the arbitrary nature of the functional response curves assumed in this model and in others like it. In other words, constant non-saturating rates of immune effector proliferation, and pathogen replication. To what extent is feedback destabilized by increasing nonlinearities in response functions? The purpose of these models is often concerned with "proof of principle", establishing the plausibility of intuitive notions of control, rather than empirical fitting of experimental data.

4.3.2 Barkai and Leibler's chemotaxis network

Feedback is no less important in regulating reactions within a cell as among populations of cells. As with variation in pathogen parameters in populations, there can as easily be variation in inputs to a cell. This means that fine-tuning parameters in advance (through evolution) to maximize a function for fixed parameters is likely to be far from robust.

Chemotaxis in bacteria describes the purposeful motion of bacteria swimming towards increasing concentrations of nutritive chemicals. Bacteria swim in alternating bouts of *smooth runs* during which they move along a single vector and *tumbling* during which they randomly reorient to a new vector. An observed property of bacterial chemotaxis is *adaptation* whereby the steady state tumbling frequency in a homogeneous chemical environment is independent of the concentration of chemical. This is a means of ensuring constant responsiveness. Barkai and Leibler (1997)ask whether feedback circuits in the putative chemotactic network are responsible for this adaptive property.

Nutritive chemical, or *ligand* L, binds to an enzymatic receptor E. The Receptor transitions between a modified and unmodified state at a rate proportional to the concentration of L denoted l. L represents the input to a cellular signal transduction system, and the concentration of active enzyme (A) interfacing with the propulsive flagellum, is the system output. An adaptive systems has the characteristic that the steady state concentration of A ($\bar{a}$) is independent of l.

The key to the robust adaptive property is to make the modification and un-modification transformation of E dependent only on the concentration of A. Yi et al (2000) point out that this adaptive property of the network is a consequence of *integral feedback control*. In mathematical terms:

$$\dot{x} = a$$

$$a = a_1 - \bar{a} = k(l - x) - \bar{a}$$

Here the time integral of the system error (x), the difference between the actual output (a_1) and the desired equilibrium output ($\bar{a}$), is fed back into the system. The parameter k is the gain of the system. In this way one obtains robust asymptotic tracking of variations in the input l.

4.4 Antiredundancy through apoptosis in neoplastic lineages

Tumorigenesis marks the onset of unregulated cell proliferation. In most long lived mammals, progress towards tumorigenesis, involves the cumulative loss of important regulatory genes monitoring the genetic state of defective cells. An important class of regulatory genes are the *tumor suppressor genes* (Levine 1993, 1997) that respond to mutations by inducing programmed cell death (apoptosis) or repairing damaged DNA. Apoptosis represents a strategy of antiredundancy or purging, in which defective cells are removed, and subsequently replaced by the descendents of healthy cells in the surrounding tissue. Purging as a mechanism of robustness thus depends crucially on population sizes large enough to allow for the replacement of eliminated cells.

Plotkin and Nowak (2002) have modeled the waiting time for dividing cells undergoing mutation and mutation-induced apoptosis to reach a tumorigenic state. Assume that L genes in the genome of dividing cells regulate healthy cell cycle function. For each cell, count the number of mutations in L and call it k. When the value of $k = n$ the cell is tumorigenic. During each cell division a cell with k mutations can divide and remain in the same state with a probability q_k or mutate with a probability $p_k = 1 - q_k$. Any cell with $k \geq 1$ mutations is under the surveillance of tumor suppressor genes and can be induced into apoptosis with a probability α_k. Apoptosis will fail with a probability $\beta_k = 1 - \alpha_k$.

These probabilities can be used to construct a Markovian model of cancer progression, with three important assumptions: (1) there are no population dynamics – cell populations are of a large fixed size with no fixation of mutant lineages, (2) symmetric mutations such that only the total number of mutations k and not the position of these mutations in a string of length L is significant, (3) the cell with n mutations is an absorbing state. With these assumptions Plotkin and Nowak write down a $(n + 1)$ x $(n + 1)$ transition matrix:

$$
\begin{array}{c} \\ 0 \\ 1 \\ 2 \\ \vdots \\ n-1 \\ n \end{array}
\begin{array}{c}
\begin{array}{cccccc} 0 & 1 & 2 & \dots & n-1 & n \end{array} \\
\left(\begin{array}{cccccc}
q_0 & p_0 & 0 & \dots & \dots & 0 \\
\alpha_1 & \beta_1 q_1 & \beta_1 p_1 & \dots & & 0 \\
\alpha_2 & 0 & \beta_2 q_2 & \beta_2 p_2 & & 0 \\
\vdots & & \ddots & \ddots & & \vdots \\
\alpha_{n-1} & 0 & \dots & 0 & \beta_{n-1} q_{n-1} & \beta_n q_n \\
0 & \dots & \dots & ... & 0 & 1
\end{array} \right)
\end{array}
$$

This is a flexible formulations as it allows for either *genomic instability* in which $\alpha_0 > \alpha_1 > ... > \alpha_{n-1}$, which describes how the incidence of mutations reduces the efficacy of the apoptotic response or when $\alpha_0 < \alpha_1 < ... < \alpha_{n-1}$ which reflects an increasing probability of cells with more mutations undergoing effective surveillance. I will only discuss the case in which $q = q_1 = q_2 = \ldots = q_{n-1}$ and $\alpha = \alpha_1 = \alpha_2 = \ldots = \alpha_{n-1}$

The effects of apoptotic purging can be demostrated by comparing the waiting time for $k = n$ of a non-apoptotic cell assuming thereby that $\alpha_i = 0$ for all i, and the alternative case with apoptosis as described above in which $\alpha_i > 0$ for all i.

The waiting without apoptosis for one cell in a tissue of N cells to obtain n mutations is given by

$$T = \frac{1}{log(1/q)(n-1)!^N} \int_0^\infty \Gamma(n,a)^N da$$

where $\Gamma(.,.)$ is the incomplete Gamma function. The waiting time for a single cell with apoptosis to obtain n mutaitons is given by,

$$T = \frac{p\beta(\alpha + p_0)}{\alpha p_0(\alpha q + p)(1 - \alpha/(p + \alpha q))^n} - \frac{1}{\alpha}.$$

In the case without apoptosis, the waiting time depends inversely on the logarithm of replication fidelity q. With apoptosis the waiting time grows exponentially with n. Thus purging of damaged cells, prolongs the waiting time to tumorigenesis, and thereby increases the latency of cancer.

4.5 Spatial compartmentalization of predators and prey: infectious disease

Theoretical immunology is in large part based on the reinterpretation of the immune system as an interaction between predators and prey. Whereas in ecology these might be carnivores and herbivores, in immunology these might be cytotoxic T cells and virus infected cells. Immune effector cells proliferate in response to antigen presented by infected cells, in which the rate of proliferation is likely to be proportional to the number of infected cells presenting antigen. The destruction of infected cells brings about a concomitant reduction in effector cell proliferation. We therefore expect oscillatory dynamics. In ecology one of the principal measures of population stability is the variance in species abundance. Large amplitude oscillations are thought to make populations vulnerable, whereas low amplitude oscillations are a sign of, robustness (May 1973). In ecology – species extinction is at stake, in immunology – a loss of effector cells and a loss of regulatory control are at stake.

Jansen and de Roos (2000) have studies the following two compartment model. Consider two populations of predators P_1 and P_2 and prey populations, N_1 and N_2. Predators are able to migrate from one compartment to another with a probability $m/2$.

$$\dot{n_1} = rn_1 - n_1 p_1$$

$$\dot{p_1} = n_1 p_1 - \mu p_1 + \frac{m}{2}(p_2 - p_1)$$

$$\dot{n_1} = rn_2 - n_2 p_2$$

$$\dot{p_1} = n_2 p_2 - \mu p_2 + \frac{m}{2}(p_1 - p_2)$$

The rate of predator proliferation is given by rp_i and the death rate μp_i. From an immunological perspective we might think of two strains of infecting virus and their corresponding T cell receptors.

Assuming equal densities of predators ($p_1 = p_2$) and prey ($n_1 = n_2$), the model reduces to the non-spatial Lotka-Volterra model, in which densities oscillate permanently at an amplitude determined by the initial conditions. However, if small differences in densities are allowed between compartments, these transiently increase with a correlated reduction in the amplitude of the oscillations in the average densities. This is because in the compartmental model, large amplitude oscillations are diffusively unstable (statistical stabilization), whereas in the single population model, oscillations of any amplitude can be maintained. Thus establishing compartments in which pathogens will be attacked (such as lymph nodes), rather than fostering the likeness of a single population, should allow organisms to limit variation in pathogen densities.

4.6 Distributed processing in the nervous system

The connectionist modeling paradigm has become the dominant theoretical framework for thinking about information processing by the nervous system (McClelland 1988, Hertz 1991). While the mapping from neural network to neural systems is highly approximate, the objective in connectionist models is to explore the properties and limits of a *"gadenkenexperiment"* in which information is distributed over a population of homogeneous, computationally trivial units. Out of this research have arisen the following robustness observations: (1) pattern recognition of corrupted inputs, (2) categorization or generalization of noisy inputs, and (3) graceful degradation in response to graded perturbations in network input or network structure. There is some sense that network models are intrinsically fault tolerant as a result of the distributed nature of the information representation. The mentioned afore principles of redundancy and modularity are likely to participate in connectionist robustness but do not exactly capture the distributed nature of the information in a neural network model.

The canonical representation of a feedforward neural network is:

$$S_i = f(\sum_j w_{ij} S_j - \theta_i)$$

where S_i is the output of unit i, w_{ij} are the weights from unit j to unit i and θ_i is the activation threshold of unit i. The function $f(.)$ is most often of the form or a non-linear squashing function or a step-function. Robustness of a network can be assessed as the deviation of the actual output vector ($\mathbf{S}$) from an desired output vector ($\mathbf{O}$). A common metric is the RMS error:

$$\epsilon = \sqrt{\frac{1}{N}\sum_i^N (S_i - O_i)^2}.$$

Perturbations in S_j or w_{ij} can then be assessed quantitatively. An lternative error function for binary or "bi-polar" units is to use the Hamming distance between $\mathbf{S}$ and $\mathbf{O}$.

4.6.1 Joanisse and Seidenberg on verb morphology

There has been some debate on whether brain injured patients have a greater difficulty in constructing the irregular past tense of familiar verbs or the regular past tense of nonsense (nonce) words. The impairment has been used to discriminate between damage to rule following (regular) versus damage to associative memories (irregular). The construction of the past tense has become a paradigmatic linguistic system for studying the difference between look up tables for exceptions and rules for common verbs (Pinker 1999). Joanisse and Seidenberg (1999) constructed a simple neural network model in which output units represent a sequence of phonological features - ordered lists of vowels (V) and consonants (C). Thus each word can be aligned with a basic template: CCVVCCC-VC. Tasted might read: C0V0CC0VC. In which "0" are wildcards or empty slots not filled by a given verb.

Each verb is represented by a unique hidden unit in the network. In addition the network contains semantic units to render verb meanings. Input units encode basic phonology as with the output units. Thus inputs connect to hidden units which connect to output units. Semantic units also connect to hidden units recurrently. One of the tasks of the network is to take a phonological input and a tense marker and generate an identical output (autoassociative mapping), another required a semantic input to be mapped onto an appropriate phonological unit.

Perturbations to the network involved severing a proportion of connections or by adding Gaussian noise to semantic units or phonological units. "Lesions" to 5% or less of the connections, had almost no effect on performance (as measured by proportion of correct outputs given a target vector - Hamming metric). Perturbations of over 5% and higher lead to a roughly linear reduction in system performance. Perturbations to the phonological units tended to produce "irregularization" errors, whereas damage to the semantic units tended to produce regularization of irregular verbs.

Thus this network was able to preserve its basic function over a small range of perturbations, above which it degraded gracefully. This linear reduction in system performance is a result of the distributed nature of the computation. Moreover, the way in which the model lost robustness, reflected in some way, the pattern of language deficit observed in Alzheimer's or Parkinsonian patients.

4.7 The extended phenotype of human culture

The derivation of human culture from genetic processes remains a controversial and often poorly-posed enterprise. However, it is possible to ask whether there are universal tendencies among human populations to institutionalize rules that minimize the impact of perturbations. In other words are there rules, norms and procedures that serve to make human populations more robust? The mathematical study of the stability of human culture to social perturbations is the domain of game theory.

One area in which the human species has been stated to be unique is in the possession of arbitrary symbols combined with a combinatorial grammar. An essential early step in the evolution of language, has been the evolution of phonological rules, in which phonemes are combined into words. Why should this transition take place. Why use compositional signals rather than expanding the number of phonemes ? Nowak and Krakauer (1999) and Nowak, Krakauer and Dress (1999) present a simple formalism of this problem, and demonstrate that one important selection pressure in favor of compositional signals is a need to become robust against errors in signal perception (Grassly et al 2000). As a result of space limitations, I shall only demonstrate the nature of the signalling problem, and omit the full solution.

Assume that a language L employs n signals to communicate about n objects. When two individuals communicate, they obtain a payoff:

$$F = \sum_{i=1}^{n} a_i$$

If all objects have the same value, then the total payoff is simply $F = kn$. In reality communication is error prone. Denote the probability of mistaking a signal i for a signal j u_{ij}. The error matrix $\mathbf{U}$ is a row stochastic error matrix. The diagonal values u_{ii} give the probability of correct communication. Hence

$$F = \sum_{i=1}^{n} a_i u_{ii}$$

The error matrix can be defined in terms of similarity between any two signals i and j: s_{ij}. Similarity is a value between 0 and 1 and hence $u_{ij} = s_{ij} / \sum_{k=1}^{n} s_{ik}$. This enables us to write the payoff in terms of signal similarity,

$$F = \sum_{i=1}^{n} \left(\frac{a_i}{\sum_{j=1}^{n} s_{ij}} \right)$$

Signals are embedded in some metric space X and d_{ij} denotes the distance between i and j. Assume that similarity is a monotonically decreasing function of distance, $s_{ij} = f(d_{ij})$. One choice of function is, $s_{ij} = exp(-\alpha d_{ij})$. Where the parameter α is a measure of the resolution of perception.

For a given number of objects we wish to find the optimum configuration of sounds $x_1, \ldots, x_n$ in a sound continuum which maximize the payoff function,

$$F = \sum_{i=1}^{n} \left(\frac{1}{\sum_{j=1}^{n} exp(-\alpha |x_i - x_j|)} \right)$$

It can be proved that the maximum value of F, as n tends to infinity, converges to:

$$F_{max} = 1 + \alpha/2$$

For any given value of perceptual accuracy α, the payoff converges to a maximum as a result of perceptual error. Increasing the number of signals increases the number of objects that can be communicated about, but at the cost of increased ambiguity. We have called this the *linguistic error limit*. It is our hypothesis that phonology, word formation and simple grammar evolved through a need for greater robustness in response to inevitable errors of communication. The key to understanding how this works is to think in terms of composite words, in which a word W_{ij} consists of phenemes i and j. The similarity between words W_{ij} and W_{kl} is given by $s_{ik}s_{jl}$. The payoff to a language that contains n^2 words to describe n^2 objects is

$$F = \sum_{i=1}^{n} \sum_{j=1}^{n} \left(\frac{1}{\sum_{k=1}^{n} \sum_{l=1}^{n} s_{ik} s_{jl}} \right)$$

$$= \left[\sum_{i=1}^{n} \left(\frac{1}{\sum_{j=1}^{n} s_{ij}} \right) \right]^2$$

and for words of length L

$$= \left[\sum_{i=1}^{n} \left(\frac{1}{\sum_{j=1}^{n} s_{ij}} \right) \right]^L$$

Hence the total payoff can now grow exponentially with the length of words. Words, according this formulation, are a cultural robustness mechanism.

5 Awaiting a synthesis of robustness in biological systems

I have presented a superficial overview of various research projects aimed at understanding robustness in biological systems. I have tried to organize this work into a number of principles of robustness - a theoretical taxonomy - in order that common patterns and mechanism, might become apparent to the reader. It is unfortunate that there does not exist a single theory of biological robustness that might be applied to these several different problems. The historical, and to some extent contingent nature of biological organization, is in large part responsible for this theoretical deficit.

There are however glimpses of intersection among principles - redundancy, modularity, spatial compartmentalization and distributed processing, share the use of a multiplicity of self-contained units discretely connected, to ensure a degree of autonomy of processing. The feedback control, the developmental module and the connectionist model all exploit saturation effects to damp down the consequences of non-linearity. Almost all the models assume some form of sparse connectivity, whether it be among neurons, classes of mutation, modules, signaling molecules, or immune effectors.

There are then some hints of meta-principles of robustness, and these to a suitably shrewd theorist, might suggest some means and direction of formal unification.

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