

The principle of sufficiency and the evolution of control: using control analysis to understand the design principles of biological systems

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Abstract

Control analysis can be used to try to understand why (quantitatively) systems are the way that they are, from rate constants within proteins to the relative amount of different tissues in organisms. Many biological parameters appear to be optimized to maximize rates under the constraint of minimizing space utilization. For any biological process with multiple steps that compete for control in series, evolution by natural selection will tend to even out the control exerted by each step. This is for two reasons: (i) shared control maximizes the flux for minimum protein concentration, and (ii) the selection pressure on any step is proportional to its control, and selection will, by increasing the rate of a step (relative to other steps), decrease its control over a pathway. The control coefficient of a parameter P over fitness can be defined as $(\partial N/N)/(\partial P/P)$, where N is the number of individuals in the population, and ∂N is the change in that number as a result of the change in P . This control coefficient is equal to the selection pressure on P . I argue that biological systems optimized by natural selection will conform to a principle of sufficiency, such that the control coefficient of all parameters over fitness is 0. Thus in an optimized system small changes in parameters will have a negligible effect on fitness. This principle naturally leads to (and is supported by) the dominance of wild-type alleles over null mutants.

Introduction

One type of question that arises in biology is: ‘why this much?’ or in more detail: ‘why are the components or parameters of a particular biological system present at the particular levels or values that they are?’. For example, why are molecular or physiological rates, amounts or ratios what they are? Why is the level of this particular protein/RNA/metabolite/signal whatever it is in this cell/organism? Why are there this many cells, or that amount of liver, or such-and-such heart rate? Why does this enzyme/transporter have these rate constants? Why does this species grow or reproduce at this rate? These are general questions essential for understanding biological systems, for changing such systems and for understanding why they go wrong in pathology.

Several different approaches have been used to address quantitative ‘why’ questions of this type. These approaches fall into three main categories: adaptation, constraints and history. According to the first approach, biological systems are the way they are because they are optimally adapted (as a result of evolution by natural selection) to perform some function necessary for survival and/or spread of the genes. An example of this type of approach to answering quantitative ‘why’ questions is the hypothesis of symmorphosis, defined as the state of structural design resulting from morphogenesis

regulated to match functional demand [1]. This proposes that the parts of a biological system are optimally designed to be just sufficient to perform their biological function and no more, because such systems are economically designed and subject to resource constraints. However, adaptationist approaches have been criticized for not taking sufficient account of constraints and history [2]. Constraint-based approaches to ‘why’ questions focus on the structural, chemical, physical or biological constraints that limit biological variables to certain values. An example of this type of approach is allometry, which generally assumes that the empirical rules of scaling of biological systems result from constraints (e.g. the brain size or metabolic rate of this species must be this due to the physics of gas exchange and surface to volume ratios) [3]. Historical approaches to ‘why’ questions focus on the evolutionary history of the variable and species in question, and may invoke the accidental/random aspects of evolutionary history or genetic drift to emphasize non-adaptive evolution [2].

In order to answer quantitative ‘why’-type questions it would be useful to have an approach that respected adaptation, constraints and history, while quantitatively linking parameter values to evolution. One potentially useful approach is MCA (Metabolic Control Analysis) and related types of sensitivity analysis. MCA quantifies the sensitivity of system properties to changes in the values of parameters within (or interacting with) the system (e.g. how much does a metabolic flux change when the level of a protein is changed). But the real power of MCA is that it gives a framework for understanding why these sensitivities and other system

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Abbreviations used: MCA, Metabolic Control Analysis.

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properties are what they are. In what follows I will illustrate some of the insights that MCA has given into the design properties of biological systems, and then go on to outline an overarching principle linking MCA to evolution: the principle of sufficiency.

Metabolic control

Within the theory of MCA (reviewed in [4]) the extent to which any component step or parameter (P) limits the steady-state flux (J) of the overall process is quantified as a flux control coefficient (C) of P over J , where:

$$C_p^J = \frac{\partial J}{J} \frac{P}{\partial P} \quad (1)$$

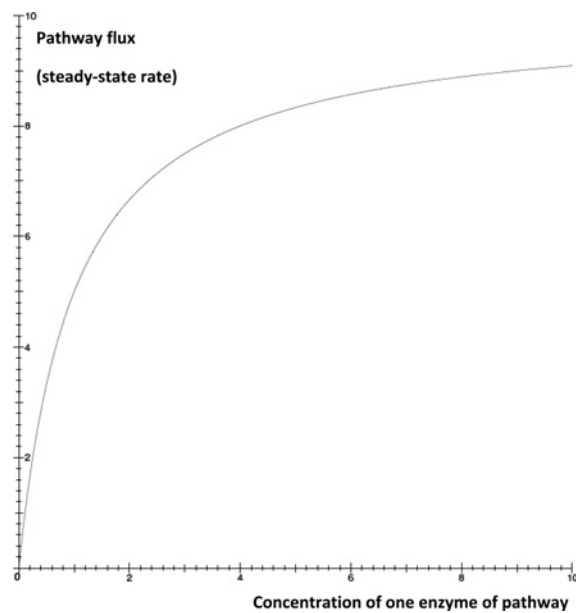
This coefficient closely approximates the percentage increase in the steady-state rate of the overall process brought about by a 1% increase in P . A step with a control coefficient of 1 is rate-limiting, whereas a step with a control coefficient of 0 has no effect on the rate of the process. An important property of these flux control coefficients is that (if the system has certain common properties) the sum of the control coefficients is equal to 1. Thus in an unbranched system if one step has a control coefficient of 1, then normally all the other steps must have control coefficients of 0. Also, if the system is not branched, the control coefficients of all components will normally lie between 0 and 1. This has further important implications: (i) if the system has multiple component steps, most of steps must have low control coefficients, and (ii) if the control coefficient of one step changes, then the control coefficient of at least one other step must change as well (if all the coefficients are to still equal 1).

We can define control coefficients for: (i) the control exerted by individual rate constants over the rates of isolated enzymes or other molecular processes [5], (ii) the control exerted by individual enzymes over the rates of isolated metabolic pathways [4], (iii) the control exerted by individual pathways over the rates of cellular processes in isolated organelles, cells or organs [6–8], and (iv) the control exerted by individual cells or organs over whole-body fluxes or processes [9]. Note that if the control coefficients are defined appropriately, then the control exerted by a rate constant within an enzyme over a whole-body process may be calculated as the product of these four control coefficients.

Experimental measurement of the control coefficients of enzymes and transporters within metabolic pathways has shown in general that several enzymes share the control over pathway rates, and thus most control coefficients are small (reviewed in [4]). This finding is contrary to the original expectation that most pathways would have a single rate-limiting step. So why is it that in general control is shared in pathways? A teleological answer to this question is that shared control is less wasteful (and therefore more efficient) in terms of resource usage. If a single enzyme in a pathway is rate-limiting, then all the other enzymes can be decreased in concentration without affecting the rate of the pathway, at least until each of these ‘excess’ enzymes becomes partially

Figure 1 | General dependence of the rate of a linear pathway on the concentration of one of its enzymes (assuming Michaelis–Menton kinetics of the enzymes)

The gradient of the line is equal to the (non-normalized) flux control coefficient of that enzyme at that concentration. Normalized flux control coefficients start at 1 at very low enzyme concentration, and decrease with increased enzyme concentration, towards 0 at very high concentrations. Activation of enzymes (by increasing V_{\max} , equivalent to increasing enzyme concentration) will in general decrease the enzyme’s control coefficient and can only cause a large increase in pathway flux if the enzyme is starting at a very low concentration (far left-hand side of this graph) with a high control coefficient. This latter condition is inefficient, because in effect all other enzymes in the pathway must be present in excess.



rate-limiting for the pathway (see Figure 1). Shared control enables a pathway to maintain a given flux at a much lower total protein concentration (or attain maximum pathway flux for a given protein concentration). In fact minimization of protein for a given flux (or maximization of flux for a given amount of protein) results when the absolute flux control coefficients ($\partial J/\partial E$, where E is the concentration of the enzyme) are equal, which means the normalized control coefficients ($\partial J/J)/(\partial E/E)$ are inversely proportional to the specific activities of each enzyme [8]. Thus this is a prediction, and potentially an explanation, of the relative concentrations of enzymes in a pathway.

Where the system under consideration is the whole body, maximization of rates (or optimization of functions) for minimum protein/cells/tissue will result in the sharing of control between component processes, because any component process that has low control is effectively ‘in excess’ and can be decreased without affecting function, and therefore is likely to be decreased by selection until

it does gain control. This is consistent with the hypothesis of symmorphosis, defined as the state of structural design resulting from morphogenesis regulated to match functional demand [1]. This hypothesis suggests that the components of a biological system will generally evolve to be just sufficient for function (and no more). It has been evoked to explain the evolutionary design of physiological processes such as maximal metabolic rate [1], as well as mitochondrial respiratory chain components [10].

Shared control has disadvantages in terms of regulation of pathways, in that it is difficult to increase the flux of the pathway substantially without activating every single enzyme to a similar extent [11,12]. This is because increasing the amount of an enzyme that is partially rate-limiting, will decrease its control coefficient, and therefore further increases will have less and less effect on the rate of the pathway (see Figure 1). A partial solution to this problem of regulating pathways with shared control is allosteric enzymes. Allosteric enzymes are activated not by increasing V_{\max} (which decreases control) but rather by decreasing K_m and switching from sigmoidal to hyperbolic kinetics, which increases the control exerted by the enzyme [13]. Thus allosteric enzymes can have low control when not activated (thus enabling shared control, which is efficient), but high control when activated (thus enabling effective regulation of pathways).

Given that shared control has disadvantages, the existence of shared control suggests that there has been strong selection pressure during evolution to maximize pathway rates and/or minimize protein levels. The advantages of maximizing pathway rates are more-or-less obvious: increased energy acquisition and production, increased speed relative to competitors, predators and prey, increased growth rates and increased reproductive rates are just some of the advantages. Pathway rates can be increased simply by increasing the levels of all the enzymes and transporters of the pathway and related pathways. However, this means of increasing pathway rates rapidly reaches a fundamental resource limit: the amount of space available per unit volume. If one keeps increasing metabolic rate by increasing concentrations of all the components, eventually there is no space left, or at least insufficient space for water and other components to mediate diffusion (and circulation). This limit was probably reached early in evolution, because essentially all living organisms are space-limited and their cellular contents are close to solubility and diffusion limits [14]. Once the space limit is reached, further increases in metabolic rates can only be achieved by: (i) maximizing the specific activity of enzymes/proteins, (ii) maximizing the specific activity of pathways by adjusting the relative levels of the different pathway enzymes, (iii) maximizing cellular metabolic rates by adjusting the relative fluxes of different pathways, and (iv) maximizing the whole-body metabolic rate by adjusting the relative amounts of different cells/tissues/organs in the body. In each case the maximization can be done by equalizing the non-normalized flux control coefficients (at least for unbranched pathways) [14].

The evolution of control

A separate explanation as to why control is shared in pathways is historical/mechanistic, rather than teleological. The selection pressure exerted on a step (e.g. an enzyme, a rate constant within an enzyme or the amount of a tissue) is equal to the control coefficient of that step over a phenotypic trait (such a pathway rate) multiplied by the control coefficient of that trait over evolutionary fitness (summed for all traits affected). Thus steps with high (positive) control over a pathway are generally going to have high (positive) selection pressure on them to increase rate (or amount). If evolution were to start from a system where a single step was rate-limiting, then only this step would have selection pressure on it to increase its rate. However, when the rate of this step was substantially increased, then it would no longer be rate-limiting (i.e. its control coefficient would decrease) because it will no longer be the slowest step (see Figure 1) [13,15]. Consequently some other step (or steps) must become partially rate-limiting, and thus these steps in turn will become subject to selection pressure to increase their rate, which in turn will cause other steps to become partially rate-limiting. Furthermore, steps that are not rate-limiting will have no selection pressure to prevent them undergoing genetic drift and deleterious mutations to forms with lower rates, thus in general they will drift to lower rates [16]. When the rate is low enough, the step will become partially rate-limiting, and therefore subject to selection pressure, preventing it falling further. Thus, during the process of evolution by natural selection to a higher rate, there is a strong tendency for most or all steps to become partially rate-limiting; in fact in the limit for the control coefficients of all steps to become equal. This is because selection pressure is only felt by those steps that are rate-limiting, and this selection tends to decrease the control exerted by that step and increase that of others; whereas steps without control have no selection pressure preventing them back-mutating to lower rates and thus increasing their control. Thus the process of natural selection itself causes an equalization of control coefficients.

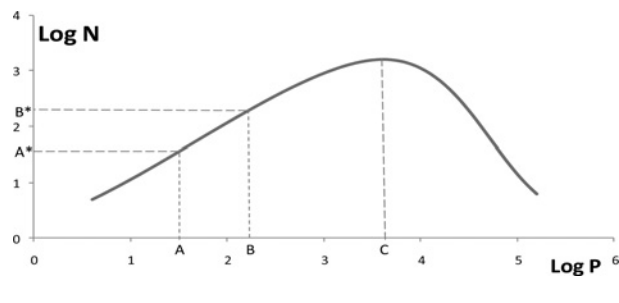
I am going to have to quantify what I mean here. The control coefficient of a parameter (P ; e.g. rate constant, amount of enzyme or cells) over evolutionary fitness can be defined as:

$$C_P^N = \frac{\partial N}{N} \frac{P}{\partial P} \quad (2)$$

where N is the number of individuals (in which P is changed), and ∂N is the change in that number caused by the change in the parameter P (usually calculated over a single generation, although in principle it could refer to the steady-state after many generations). Changes in N result from selection on survival or reproduction. When C_P^N is larger than 0, increases in P cause the number of individuals (with that increase in P) to increase; when C_P^N is smaller than 0, increases in P cause

Figure 2 | Hypothetical dependence of population size (N) on some parameter (P)

The gradient of this log-log plot at any point on the curve is the control coefficient of the parameter over fitness, which is also equal to the selection pressure on that parameter. If the size of the parameter P varies within the population from A to B , with corresponding populations after selection of A^* and B^* , then the population of individuals with parameter values close to B will increase over time relative to those with parameters close to A . Thus if parameter values continue to vary around the mean, then the population will eventually evolve to parameter value C , where fitness is maximal and the control coefficient of P over fitness (selection pressure on P) is 0. Beyond C , the selection pressure on P is negative, so that any population in this zone will evolve back towards C (assuming P can vary).



the number of individuals to decrease over time (generations); when C_p^N is 0, changes in P have no effect.

Importantly, C_p^N is also equal to the selection pressure on parameter P . The selection pressure quantifies the extent to which a change in parameter P affects fitness, i.e. survival of the genotype. A parameter with a strong positive-selection pressure on it, will result in a large increase in fitness (i.e. survival/spread of the genotype) if it is increased; thus, if P varies in the population, larger values of P will automatically be selected. A parameter with a negative C_p^N will decrease fitness if increased, and thus P will automatically decrease over generations of selection. A parameter with 0 or negligible C_p^N has no selection pressure on it, and thus is free to vary with no effect on fitness.

If there is some biological parameter (P), which varies between genotypes, and that variation affects fitness ($C_p^N \neq 0$), then (by the above definition of C_p^N) after a generation genotypes bearing a more optimal value of P will have increased (and those with a less optimal genotype will have decreased). If the parameter P has an optimal value (in the sense of a local maximal value beyond which fitness falls), and if P changes/varies in the population as a result of genetic mutation/change, then the automatic result of selection on P over generations is for P to evolve to a local optimum (where $C_p^N = 0$). Thus by exploring the local parameter space, evolution by natural selection will reach a local optima where $C_p^N = 0$ (see Figure 2). Of course, if the environment has changed recently, the species/organism may not be optimized for that environment, but this is likely to be a relatively rare occurrence and even then apply to only relatively few parameters of an organism.

Thus if a population or species has survived long enough in a stable environment to be optimized for that environment (i.e. explored the local parameter space such that the current value of P outcompetes larger or smaller values), then that population should conform to a ‘principle of sufficiency’. The principle of sufficiency states that the components or parameters (P) of biological systems are present at the optimum level for biological fitness, such that $C_p^N = 0$. Thus small changes in the parameter will have no effect on fitness, large increases or decreases in the parameter will decrease fitness. The principle is a bit like the Goldilocks principle: things are not too hot, or not too cold, but just right. The principle seems innocuous, but actually is very useful – if it is true. The evidence that it is true is that, in most cases, heterozygote null mutants or hemizygotes have the same phenotype as the wild-type, i.e. halving the amount of a protein has no detectable effect on the organism [17]. Thus the dominance of the wild-type allele over the mutant allele is evidence for the principle of sufficiency, but this principle could be used as an explanation for dominance. Populations automatically evolve to a local optimum of P where $C_p^N = 0$, so for example if P is the amount of a gene product/protein, then at that local optimum, fitness is insensitive to the amount of the protein, and therefore to the gene dosage, i.e. the wild-type allele is haploinsufficient. The principle of sufficiency also optimizes robustness, i.e. when $C_p^N = 0$ fitness is insensitive to changes in parameter P .

If the sensitivity of fitness to most parameters is 0 ($C_p^N = 0$), then why are the control coefficients of steps in linear pathways over pathway flux normally positive (shared control with the sum of control equal to 1)? The effect of changing a parameter on fitness C_p^N can be decomposed into C_p^J the effect of changing the parameter on some trait (e.g. a pathway flux J), and C_J^N the effect of changing the trait/pathway J on fitness, where:

$$C_p^N = C_p^J \cdot C_J^N \quad (3)$$

if P only affects one trait. However, if P affects (n) multiple traits:

$$C_p^N = \sum_{J=1}^n C_p^J \cdot C_J^N \quad (4)$$

Thus, for example, increasing the concentration of an enzyme may increase the flux of the pathway ($C_p^J > 0$) but have no effect on fitness ($C_p^N = 0$) either because the pathway flux has no effect on fitness ($C_J^N = 0$) or because the increase in the enzyme concentration also affected some trait K (other than J) which has a negative effect on fitness ($C_K^N < 0$, which cancels out the positive effect via J). In the example given, the increase in enzyme concentration may have a negative effect on fitness either (i) because it takes resources from other cellular components (such as space, amino acids and energy), or (ii) because the pathway flux may have both positive and negative effects on fitness because it both provides resources to the cell (its products) and removes resources (its substrates).

Overall the principles elucidated here may be used to try to understand why the parameters of biological systems are what they are.

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