Extending the method of mathematically controlled comparison to include numerical comparisons

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Received on December 12, 1999; revised on March 28, 2000; accepted on May 15, 2000

Abstract

Motivation: The method of mathematically controlled comparison has been used for some time to determine which of two alternative regulatory designs is better according to specific quantitative criteria for functional effectiveness. In some cases, the results obtained using this technique are general and independent of parameter values and the answers are clear-cut. In others, the result might be general, but the demonstration is difficult and numerical results with specific parameter values can help to clarify the situation. In either case, numerical results with specific parameter values can also provide an answer to the question of how much larger the values might be. In contrast, a more ambiguous result is obtained when either of the alternatives can have the larger value for a given systemic property, depending on the specific values of the parameters. In any case, introduction of specific values for the parameters reduces the generality of the results. Therefore, we have been motivated to develop and apply statistical methods that would permit the use of numerical values for the parameters and yet retain some of the generality that makes mathematically controlled comparison so attractive.

Results: We illustrate this new numerical method in a step-by-step application using a very simple didactic example. We also validate the results by comparison with the corresponding results obtained using the previously developed analytical method. The analytical approach is briefly present for reference purposes, since some of the same key concepts are needed to understand the numerical method and the results are needed for comparison. The numerical method confirms the qualitative differences between the systemic behavior of alternative designs obtained from the analytical method. In addition, the numerical method allows for quantification of the differences and it provides results that are general in a statistical sense. For example, the older analytical method showed that overall feedback inhibition in an unbranched pathway makes the system more robust whereas it decreases the stability margin of the steady state. The numerical method shows that the magnitudes of these differences are not comparable. The differences in stability margins (1–2% on average) are small when compared to the differences in robustness (50–100% on average). Furthermore, the numerical method shows that the system with overall feedback responds more quickly to change than the otherwise equivalent system without overall feedback. These results suggest reasons why overall feedback inhibition is such a prevalent regulatory pattern in unbranched biosynthetic pathways.

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Introduction

The experimental investigation of biological regulatory mechanisms has revealed an enormous variety of alternative molecular designs and raised questions about their function, design and evolution. Mathematically controlled comparison is a technique that was specifically developed to study such alternative regulatory designs (Savageau, 1972). By using the mathematical analog of a well-controlled experiment, this technique analytically determines the irreducible qualitative differences in the systemic behavior of the alternative designs. This technique has been used to study alternative regulatory designs in metabolic pathways (e.g. Savageau, 1974; Hunding, 1974; Savageau and Jacknow, 1979), in gene circuits (e.g. Hlavacek and Savageau, 1996), in immune
networks (e.g., Irvine and Savageau, 1985; De Boer and Hogeweg, 1989a,b), and in the host-pathogen response to HIV infection (De Boer and Perelson, 1998). The introduction of numerical values for the parameters provides quantification of these differences in specific cases but eliminates the generality of the results. In this paper we introduce a numerical approach to mathematically controlled comparisons that allows the introduction of specific numerical values for the parameters in the analysis while still retaining the generality of the results in a statistical sense.

The most common use of mathematically controlled comparison requires the existence of closed-form solutions for the steady state. Such solutions can be obtained by using the local S-system representation to characterize the pathway of interest. Important functional constraints are introduced by equating relevant steady-state properties of the alternative systems being compared. Further analysis (dynamic as well as steady state) is performed and a profile of ratios for corresponding results from the alternative systems is constructed. In some cases, a ratio can be determined analytically to be less than, equal to, or greater than unity. For example, if the ratio of values for property P in a reference system to the same property in an alternate system is larger than unity, then the reference system can always be made to have a larger value for P no matter how large the value for P in the alternate system.

However, if one wishes to know how much greater than unity a given ratio is, then one needs to examine actual values for the parameters. These parameter values are not always available or, if available, are not always accurate. Moreover, there are cases in which the ratio can be less than or greater than unity depending upon the specific values for the parameters. In any case, the results of such a numerical comparison are no longer general. In this work we propose a novel approach to this problem that combines the method of mathematically controlled comparison with statistical techniques (Alves and Savageau, 2000a,b) to yield numerical results that are general in a statistical sense.

Although we could describe the numerical method in general terms, this approach would be too abstract and difficult to understand. Instead, we will illustrate this new numerical method by means of a step-by-step application using a very simple didactic example. We also validate the results by comparison with the corresponding results obtained using the previously developed analytical method. The analytical approach is briefly presented for reference purposes, because some of the same key concepts are needed to understand the numerical method and because the results are needed for comparison.

**Methods**

*Alternative models*

The didactic example that we use to illustrate our numerical method is an unbranched three-step pathway as shown schematically in Figure 1. This is an abstraction from actual three-step biosynthetic pathways such as those involved in the biosynthesis of amino acids (e.g. http://www.genome.ad.jp/kegg/dblinks/map/map01150.html). The independent variable X4 represents the cell’s demand for the end product X3. If the cell requires large amounts of X3, then the value of X4 will be high; if small amounts of X3 are required, then the value of X4 will be low. These models show the pathway with and without end-product inhibition (Umberger, 1956; Yates and Pardee, 1956; Monod et al., 1963), a common feature of such pathways. We have observed (by consulting the database at http://www.genome.ad.jp/kegg/dblinks/map/map01150.html) that there is usually no other feedback to the first step of the pathway. However, feedback to intermediate reactions may exist and for this reason we consider models with all possible intermediary feedback interactions.

**Differential equations**

The dynamic behavior of each model can be described by a set of ordinary differential equations, one equation per
intermediate. This set of equations can be approximated to the first order in logarithmic space, yielding the canonical form for the local S-system representation (Savageau, 1969, 1996). For the model in Figure 1a this equation set becomes:

\[
\begin{align*}
dX_1/dt &= \alpha_1 X_0^{R_{10}} X_3^{R_{13}} - \alpha_2 \prod_{j=1}^{3} X_j^{R_{2j}} \\
dX_2/dt &= \alpha_2 \prod_{j=1}^{3} X_j^{R_{2j}} - \alpha_3 \prod_{j=2}^{3} X_j^{R_{3j}} \\
dX_3/dt &= \alpha_3 \prod_{j=2}^{3} X_j^{R_{3j}} - \alpha_4 X_3^{R_{43}} X_4^{R_{44}}
\end{align*}
\]

For the model in Figure 1b the equation set becomes:

\[
\begin{align*}
dX_1/dt &= \alpha_1 X_0^{R_{10}} - \alpha_2 \prod_{j=1}^{3} X_j^{R_{2j}} \\
dX_2/dt &= \alpha_2 \prod_{j=1}^{3} X_j^{R_{2j}} - \alpha_3 \prod_{j=2}^{3} X_j^{R_{3j}} \\
dX_3/dt &= \alpha_3 \prod_{j=2}^{3} X_j^{R_{3j}} - \alpha_4 X_3^{R_{43}} X_4^{R_{44}}
\end{align*}
\]

The multiplicative parameters (rate constants), \( \alpha \), influence the time scales of the reactions and are always positive. The exponential parameters (kinetic orders), \( g \), represent the influence of each metabolite on each aggregate rate law. If \( X_i \) influences the aggregate rate law \( V_j \), either as a substrate or a modulator, and if an increase in the concentration of \( X_i \) causes an increase in the rate \( V_j \), then the kinetic order will be positive. If an increase in the concentration of \( X_i \) causes a decrease in the rate \( V_j \), then the kinetic order will be negative. If an increase in the concentration of \( X_i \) causes neither an increase nor a decrease in the rate \( V_j \), then the kinetic order will be zero. Thus, the positive kinetic orders in Equation (1) are \( g_{i+1, i} \) (\( 0 \leq i \leq 3 \)), which are the kinetic orders for substrates of reactions, and \( g_{44} \), which is a scale factor arbitrarily set equal to 1.0. The remaining kinetic orders are negative, since these represent negative feedback interactions.

The temporal responsiveness of each model can be determined by perturbing the system variables, solving the corresponding dynamic equations, and calculating the time for the dependent variables to settle within 1% of their final steady-state values.

**Steady-state solution and key systemic properties**

At the steady state, which can be analytically determined, both the production and consumption terms have identical values. One can write the following matrix equation (Savageau, 1969):

\[
\begin{bmatrix}
 b_1 - g_{10} Y_0 \\
 b_2 \\
 b_3 + Y_4
\end{bmatrix} =
\begin{bmatrix}
 a_{11} & a_{12} & a_{13} \\
 a_{21} & a_{22} & a_{23} \\
 a_{31} & a_{32} & a_{33}
\end{bmatrix}
\begin{bmatrix}
 Y_1 \\
 Y_2 \\
 Y_3
\end{bmatrix}
\]

\[\mathbf{B} = \mathbf{AY}\]  

(3)

where \( b_l = \ln(\alpha_{l+1}/\alpha_l) \), \( a_{ij} = g_{ij} - g_{i+1,j} \) and \( Y_l = \ln(X_l) \). Equation (3) is linear and therefore easily solved to obtain the steady-state values for each \( Y_l \); the corresponding values for each \( X_i \) are then obtained by simple exponentiation.

Two types of coefficients, logarithmic gains and parameter sensitivities, can be used to characterize the steady state of such models (Savageau, 1971). Logarithmic gains measure the relative influence of each independent variable on each dependent variable of the model. For example,

\[
L(X_i, X_0) = \frac{d \text{Log}(X_i)}{d \text{Log}(X_0)} = \frac{d Y_i}{d Y_0}
\]

(4)

measures the percent change in the concentration of intermediate \( X_i \) caused by a percentage change in the concentration of the initial substrate \( X_0 \). Logarithmic gains provide important information concerning the amplification or attenuation of signals as they are propagated through the system.

Parameter sensitivities measure the relative influence of each parameter on each dependent variable of the model. For example,

\[
S(X_i, p_j) = \frac{d \text{Log}(X_i)}{d \text{Log}(p_j)} = \frac{d Y_i}{d p_j}
\]

(5)

measures the percent change in the concentration of intermediate \( X_i \) caused by a percentage change in the value of the parameter \( p_j \). Parameter sensitivities provide important information about system robustness, i.e., how sensitive the system is to perturbations in the parameters that define the structure of the system.

Since steady-state solutions exist in closed form we can calculate each of the two types of coefficients simply by taking the appropriate derivatives. Although the mathematical operations involved are the same in each case, it is important to keep in mind that the biological significance of the two types of coefficients is very different.

The local stability of the steady state can be determined by applying the Routh criteria (Dorf, 1992). The magnitude of the two critical Routh conditions can be used to quantify the margin of stability (Hlavacek and Savageau, 1996).
Responsiveness

Systems should respond quickly to changes in their environment. To evaluate temporal responsiveness, perturbations of 20% were made in the steady-state values of the intermediate concentrations and the time required for them to settle within 1% of their final steady-state values was then calculated. This also gives an indication of the transient time for metabolites in the system. These transit times should be low. There is no exact way to determine the transient time analytically. Thus, this part of the analysis will be dealt with only in the numerical section of the results.

Generation of random ensembles

The analytical results give qualitative information that characterizes the role of overall feedback in the system of Figure 1A. To obtain quantitative information, one must introduce specific values for the parameters and compare systems. For this purpose we have randomly generated a large ensemble of parameter sets and selected 5000 of these sets that define systems consistent with various physical and biochemical constraints. These constraints include mass balance, low concentrations of intermediates and small changes in their value to minimize the utilization of the solvent capacity in the cell, small values for parameter sensitivities so as to desensitize the system to spurious fluctuations affecting its structure, and stability margins large enough to ensure local stability of the systems. A detailed description of these methods can be found in Alves and Savageau (2000b). MathematicaTM (Wolfram, 1997) was used for all numerical procedures.

Density of ratios plot

To interpret the ratios that result from our analysis we use Density of Ratios plots as defined in Alves and Savageau (2000a). The primary density plots from the raw data have the magnitude for some property of the reference model on the x-axis and the corresponding ratio of magnitudes (reference model to alternative model) on the y-axis. The primary plot can be viewed as a list of 5000 paired values that can be ordered with respect to the reference magnitude to form a list $L_1$ in which the first pair has the lowest measured value for property $P$ in the reference model, the second has the second lowest, and so on. Secondary density plots are constructed from the primary plots by the use of moving quantile techniques with a window size of 500. The procedure is as follows. One collects the first 500 ratios from the list $L_1$, calculates the quantile of interest for this sample, and pairs this number with the median value of the corresponding $P$ values of the reference model denoted $(P)$. One advances the window by one position, collects ratios 2 to 501, calculates $(R)$, and pairs it with the corresponding $(P)$ value and continues in this manner until the last ratio from the list $L_1$ was used for the first time (for further explanation of moving median techniques see, e.g. Hamilton, 1994). The slope in the secondary plot measures the degree of correlation between the quantities plotted on the x- and y-axes. This technique is also used to examine correlations between ratios of interest and other magnitudes shared by the two systems, e.g. the correlation between the ratio of stability margins and the magnitude of a rate constant common to the two systems (for traditional applications of correlation analysis see Wherry, 1984).

Analytical comparison

Firstly, we shall exemplify the analytical aspects of a mathematically controlled comparison aimed at discovering the advantages, if any, brought about by overall feedback inhibition. This will serve to introduce key concepts that will be needed for the numerical aspects in the following section. Also, the results will be used for later comparison to validate the results obtained with the new numerical method.

We compare the systemic behavior of the model in Figure 1A (reference model) with that in Figure 1B (alternative model). To ensure that the results are due solely to the differences in design and not reversible by a mere change in parameter value, we shall insist on the following mathematical controls.

Internal and external equivalence

Only the first step in the pathway is allowed to differ between the reference model and the alternative. Therefore, to establish an internal equivalence (Savageau, 1972, 1976; Irvine, 1991) between the two designs, we require the values for the corresponding parameters of all other steps in the two models to be the same.

The first step of the pathway differs between the reference model and the alternative. If we reason that loss or gain of an inhibitory site on the first enzyme comes about by mutation, and that this mutation can cause changes in all the parameters of the process, then (taking the model in Figure 1A as reference) a mutation causing loss of overall feedback inhibition would change the parameters $g_{10}$, $g_{13}$ and $a_1$ in Equation (1) to $g'_{10}$, $g'_{13} = 0$ and $a'_1$ in Equation (2). Since we wish to determine the effects that are due solely to changes in the structure of the system, we shall specify new values for $g'_{10}$ and $a'_1$ that minimize all other effects. This can be accomplished by deriving the mathematical expression for a given steady-state property in each of the two models, equating these expressions, and then solving the constraint equation for the value of a primed parameter. For example, if we derive expressions for the logarithmic gains and require that $L(X_i, X_0)_A = L(X_i, X_0)_B$, then this equation can be solved to determine the following value for $g'_{10}$. 
Similarly, we can derive expressions for the steady-state concentrations and require that \( [X_i]_A = [X_i]_B \), then this equation can be solved to determine the following value for \( a'_1 \):

\[
\log[a'_1] = \frac{g_{43} \log[\alpha_1] + g_{13} \log[\beta_3]}{g_{43} - g_{13}}
\]

These particular values for the primed parameters make the steady-state flux, each of the corresponding steady-state concentrations, and the logarithmic gains in each of these quantities the same in both models. The process we have just described determines the maximal degree of external equivalence between the two models. There are no more 'free' parameters that can be used to reduce the differences and all remaining differences can be attributed to the change in system structure, i.e. to overall feedback inhibition. The external equivalency conditions we require insure that both the reference and the alternative models have the same steady-state concentrations and rates. This implies that the steady-state thermodynamic potential across each corresponding reaction is the same in the reference and the alternative models. Having established the conditions for maximal equivalence, we can now analyze the two models and determine their remaining differences.

**Pathway gain**

The logarithmic gains in concentrations and fluxes with respect to changes in the initial substrate \( X_0 \) determine whether the pathway is amplifying or homeostatic. When comparing pathways designed to amplify biochemical signals it is important that the alternatives provide the same high logarithmic gain. Conversely, when comparing pathways designed to attenuate biochemical signals it is important that the alternatives have the same low logarithmic gain. The method of mathematically controlled comparison insures that both the compared models will have the same logarithmic gains and thus have the same amplifying or homeostatic characteristics.

The logarithmic gains in concentration with respect to changes in demand (represented here by changes in the modulator \( X_4 \)) are smaller in the reference system, whereas the logarithmic gain in flux with respect to changes in \( X_4 \) is larger in the reference system. In this aspect, the reference system is more efficient because it can produce greater increases in flux with smaller increases in concentration. When the logarithmic gain in flux with respect to changes in demand is zero (as is the case in the alternative model), changes in demand have no influence over the flux. Thus, overall feedback inhibition makes the system better equipped to deal with changes in the demand for \( X_3 \). These results are shown in Table 1.

### Table 1. Analytical expressions for the ratios of corresponding systemic properties of the reference system and the alternative system

<table>
<thead>
<tr>
<th>Systemic property</th>
<th>( x_1 )</th>
<th>( x_2 )</th>
<th>( x_3 )</th>
<th>( v )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( L(x, X_0) )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>( L(x, X_4) )</td>
<td>B</td>
<td>C</td>
<td>A</td>
<td>1/0^a</td>
</tr>
<tr>
<td>( S(x, \alpha_1) )</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>( S(x, \alpha_2) )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>( S(x, \alpha_3) )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>( S(x, \alpha_4) )</td>
<td>B</td>
<td>C</td>
<td>A</td>
<td>1/0</td>
</tr>
<tr>
<td>( S(x, g_{10}) )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>( S(x, g_{13}) )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>( S(x, g_{32}) )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>( S(x, g_{33}) )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>( S(x, g_{43}) )</td>
<td>B</td>
<td>C</td>
<td>A</td>
<td>1/0</td>
</tr>
</tbody>
</table>

^a The three critical ratios are given by the following analytical expressions:

\[
A = 1 + \frac{g_{13}}{g_{43} - g_{13}} < 1
\]

\[
B = 1 + \frac{g_{13}(g_{32}(g_{33} - g_{43}) + g_{32}(g_{43} - g_{33}))}{(g_{43} - g_{33})g_{32}(g_{33} - g_{33})} < 1
\]

\[
C = 1 + \frac{g_{13}(g_{43} - g_{33})}{g_{33}(g_{43} - g_{43})} < 1
\]

^b The ratio 1/0 represents the division of any non-zero number by zero.

**Robustness**

The system should be robust, i.e. insensitive to fluctuations in the parameter values (Shiraishi and Savageau, 1992). This means that the sensitivity profile should, in general, be as low as possible. Whenever the sensitivity of a concentration to a parameter is different in the two models, it is smaller in the reference model, i.e. the ratio \( S(X_i, p_j)_A/S(X_i, p_j)_B \) is always less than or equal to unity. Thus, overall feedback inhibition makes each intermediate concentration less sensitive (i.e. more robust) with respect to fluctuations in parameter values.

Most of the corresponding flux sensitivities are equal in the reference and alternative models. The sensitivity \( S(V, \alpha_1) \) is smaller in the reference model, which makes this model less sensitive to changes in the molecular activity of the first enzyme, whereas the sensitivities \( S(V, g_{43}) \) and \( S(V, \alpha_4) \) are larger in the reference system because they also can reflect changes in demand. These results are shown in Table 1.

**Stability**

The steady state of the system should be stable, i.e. the system should return to its original steady state after a small perturbation. If this does not occur, the system is dysfunctional. The margins of stability for a system can be measured using the Routh criterion (Hlavacek and
Numerical controlled comparisons

Savageau, 1996). The larger these margins, the further from the boundaries of instability the system will be. The results of the analysis are as follows:

$$\frac{\text{Criterion}#_1^A}{\text{Criterion}#_1^B} = \frac{\text{Criterion}#_2^A}{\text{Criterion}#_2^B} = \frac{\text{Criterion}#_3^A}{\text{Criterion}#_3^B} = D < 1$$

where

$$D = 1 + \frac{F_1 F_2 F_3 g_{13} g_{21} g_{32}}{F_1^2 F_2^2 g_{21} g_{32} - F_1 F_2^2 g_{21} g_{32}}$$

$$- F_1^2 F_3^2 g_{21} g_{32} + F_2^2 F_3^2 g_{21} g_{32}$$

$$+ F_1 F_2^2 g_{21} g_{32} - F_2 F_3^2 g_{21} g_{32}$$

$$- F_1 F_2^2 g_{21} g_{32} + 2 F_1 F_2 g_{21} g_{22} g_{32}$$

$$- F_2 F_3^2 g_{21} g_{32} - 2 F_1 F_2 g_{21} g_{32}$$

$$+ F_1 F_2^2 g_{21} g_{32} + F_2 F_3^2 g_{21} g_{32}$$

$$+ F_1 F_2^2 g_{21} g_{32} - F_2 F_3^2 g_{21} g_{32}$$

$$+ F_1 F_2^2 g_{21} g_{32} + F_2 F_3^2 g_{21} g_{32}$$

$$- 2 F_2 F_3 g_{21} g_{32} + F_3 g_{21} g_{32}$$

$$+ F_1 F_2^2 g_{21} g_{32} - F_2 F_3^2 g_{21} g_{32}$$

$$+ F_1 F_2^2 g_{21} g_{32} + F_2 F_3^2 g_{21} g_{32}$$

$$+ F_1 F_2^2 g_{21} g_{32} - F_2 F_3^2 g_{21} g_{32}$$

$$+ F_1 F_2^2 g_{21} g_{32} + F_2 F_3^2 g_{21} g_{32}$$

$$+ F_1 F_2^2 g_{21} g_{32} - F_2 F_3^2 g_{21} g_{32}$$

with $F_i$ being the turn-over number of the pool $X_i$.

Note that the negative signs in this expression always precede parameters that represent negative feedback and consequently all terms have positive values. Thus, the reference and the alternative models differ in only one of the three Routh criteria applicable for a three variable system, and the alternative system has the larger margin of stability.

Summary

The analytical comparison gives qualitative results that characterize the role of overall feedback inhibition in the model of Figure 1A. This analysis demonstrates that the model with overall feedback inhibition is more robust and that its flux is more responsive to changes in demand for the end product, although this model has a smaller margin of stability. However, this analysis does not tell us how much more robust or how much more responsive to demand the reference model is, nor does it tell us how much smaller its margin of stability is. For answers to these questions we must consider specific values for the parameters and employ statistical techniques if we are to uncover general tendencies.

Numerical comparison

The techniques described in Alves and Savageau (2000b) have been used to generate an ensemble of 5000 parameter sets that characterize and reference the alternative systems with stable steady states. Each of these parameter sets was then inserted into the appropriate equations to determine the magnitude of the quantitative differences between reference and alternative systems.

Ratios of systemic properties

Figure 2A shows a typical Density of Ratios plot for an individual parameter sensitivity. One can clearly see that $S(X_i, p_j)_A/S(X_i, p_j)_B \leq 1$. Figure 2C shows a typical example of a Density of Ratios plot for the aggregate parameter sensitivities of a concentration variable. The aggregate parameter sensitivity of $X_i$, $S(X_i)$, is defined as the Euclidean norm of the vector whose coordinates are the sensitivities with respect to the individual parameters. [The numerical method makes it possible to use different functions of the parameter sensitivities to define an aggregate sensitivity, e.g. a weighted average of the sensitivities could be used when one knows the relative importance of the individual parameters in the model.] The ratio is defined as the aggregate parameter sensitivity in the reference model divided by the corresponding aggregate in the alternative model. Again, we see that the reference model has smaller sensitivities.

A comparison of the models on the basis of the 3rd Routh criterion for stability (Figure 2E) shows that the margin of stability is smaller for the reference model; however, the magnitude of the difference is very small with ratios always greater than 0.81. The modal class of this ratio is the one closest to 1 (defined has 0.995 < ratio < 1), with more than 35% of the models. Thus, models with or without overall feedback inhibition have very similar stability boundaries. This indicates that local stability is probably not an important criterion in comparing the models, since they are very similar in this aspect. Figure 2G shows that the model with overall feedback inhibition is typically more responsive than the alternative model lacking this inhibition, although there are a few exceptions.

Statistical analysis of ratios

Figures 2B, D, F and 2H show the moving median plots (Alves and Savageau, 2000a) corresponding to the raw Density of Ratios plots in Figures 2A, C, E and 2G. As was mentioned previously, robust systems function more reproducibly. Figure 2B shows an example of a moving median plot for an individual parameter sensitivity. There are two regions in which there is no correlation between the sensitivity in the reference model and the ratio of sensitivities in the reference and alternative models. These two regions are separated by a region with a sharp change.
in the average value of the ratio. For most other parameter sensitivities, the ratio changes less abruptly. The moving median plot for the aggregate parameter sensitivities (Figure 2D) shows that as the sensitivity increases (i.e. the robustness decreases) the ratio also tends to increase, until it reaches a limit median value. For highly robust models,
Fig. 3. Histograms comparing properties that differ between the reference system and the alternative system in Figure 1. In the left-hand panels (A, C, E, G and I) the histogram of the relevant property for the reference system is represented by a thick line whereas the same histogram for the alternative system is represented by a thin line. In the right-hand panels (B, D, F, H and J) the histogram for the ratio is represented with a thick line. (A) and (B) aggregate sensitivity of $X_1$, (C) and (D) aggregate sensitivity of $X_2$, (E) and (F) aggregate sensitivity of $X_3$, (G) and (H) 3rd Routh condition, (I) and (J) transient time.
the difference in robustness between the reference and the alternative model tends to be bigger. Thus, for models that are optimized with regard to robustness, on average, the reference model will be much more robust than the alternative model.

Figure 2F shows that the stability margin of the alternative model is always greater than that of the reference model, although on average the differences are insignificant. Hence, the stability margins are essentially the same.

As for the transient behavior of the models (Figure 2H), we can see that the responsiveness of the reference model is almost always better than or equal to that of the alternative model (more than 98% of all cases). Overall feedback inhibition has an important effect in making the model respond more quickly to perturbations in its state. Since there is no analytical expression for the transient behavior, the only way to obtain these comparative results is through the use of numerical methods.

A different way to observe that the parameter sensitivities are indeed larger in the alternative model, is by comparing histograms of corresponding sensitivities that differ between the reference and the alternative model and by plotting the histograms of the ratios directly (Figure 3). The alternative model clearly has more parameter sensitivities in the higher range of values. This approach also shows that the transient times are longer for the alternative model, whereas the difference in distributions for the stability margin is less notable. In each case, the histogram of ratios shows that the magnitudes are larger in the alternative model.

**Correlations**

The previous paper (Alves and Savageau, 2000b) has shown how different properties of the model represented in Figure 1A are correlated. Here we use the same technique to show how the differences between reference (Figure 1A) and alternative (Figure 1B) models are correlated with various steady-state properties. The differences we shall examine are the four analytical determined ratios shown in Table 1 (A–D) plus the ratio of transient times that we determine numerically (E). For each of the five ratios we plot \( \langle R \rangle_{0.05} \) as a function of \( \langle P \rangle \), where \( \langle R \rangle_{0.05} \) is the 5% quantile of ratio \( R \) and \( \langle P \rangle \) is the moving median of the steady-state property of interest. We present results for \( i = 0.05 \), \( i = 0.5 \) and \( i = 0.95 \). The moving window size used in the calculations is 500. The generic shapes of the correlation curves are shown in Figure 4, and the results of the correlation analysis are summarized with reference to these shapes in Table 2.

Each moving quantile curve for the same \( R \), \( \langle R \rangle_{0.05} \), represents a contour that shows how a given quantile of \( R \) is correlated with a particular magnitude of interest. By building a contour plot with several different quantiles, we can empirically evaluate the quality of the predicted correlation, as well as obtain non-parametric confidence interval curves for the moving median.

An example of such a contour plot is presented in Figure 5 for the correlation between the different moving quantiles of the ratio \( B \) (from Table 1) and the 2nd Routh condition for local stability. The plot gives information about the dispersion of \( B \) as a function of the 2nd Routh criterion. This dispersion decreases as the value of the Routh criterion increases. At low values of the Routh criterion the 5% quantile of \( B \) is very close to −1 and the 95% quantile is very close to 1, whereas for high values of the stability margin the 5% quantile is about −0.6 and the 95% quantile is about 0.7. In plots involving other quantities, the dispersion may increase or remain unchanged as the quantity on the x-axis increases.

The second type of information one can extract from Figure 5 regards the quality of the predicted correlation between \( B \) and the 2nd Routh criterion. From the moving quantile plot involving \( Q_{0.5} \) we determine that, on average, there is no correlation between \( B \) and the value for the 2nd Routh criterion. The other moving quantile curves show that, for high values of the stability margin, this absence of correlation is maintained for all moving quantiles. However, in the region of low values for the stability margin, the correlation is weakened.

![Fig. 4. Qualitatively different shapes for the correlation curves between different systemic properties.](image-url)
Numerical controlled comparisons

Table 2. Correlation between the five critical ratios and various systemic properties

<table>
<thead>
<tr>
<th>Systemic property</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
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<tr>
<td></td>
<td>$Q_{0.05}$</td>
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<td>$Q_{0.95}$</td>
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<td>C7</td>
<td>C8</td>
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<td>C2</td>
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<tr>
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<td>C8</td>
<td>C8</td>
<td>C9</td>
<td>C2</td>
</tr>
<tr>
<td>$V$</td>
<td>C4</td>
<td>C4</td>
<td>C4</td>
<td>C4</td>
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<tr>
<td>$L(X_1, X_0)$</td>
<td>C7</td>
<td>C7</td>
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<tr>
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<tr>
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<td>C3</td>
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</tr>
<tr>
<td>$L(X_2, X_4)$</td>
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<td>C4</td>
<td>C4</td>
<td>C9</td>
<td>C2</td>
</tr>
<tr>
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<td>C7</td>
<td>C9</td>
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<td>C9</td>
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<tr>
<td>$S(X_1)$</td>
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<td>C1</td>
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<td>C2</td>
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<td>3rd Routh</td>
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<td>C2</td>
<td>C2</td>
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<td>C3</td>
<td>C2</td>
<td>C7</td>
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</table>

$^a$ The expressions for the critical ratios A, B, and C are given in the footnotes of Table 1. The expression for ratio D is given following Equation (8) in the text. Ratio E is the ratio of transient times, which are determined numerically.

$^b$ The C values refer to the shape of the curves in Figure 5. We present the shape of the curves with a 90% confidence interval. For example, the correlation of ratio B with $L(X_1, X_4)$ has a shape C2 but its 90% boundaries show that this form can change (smoothly) between C9 and C4.

margin, a positive correlation between the stability margin and $B$ starts to develop as the quantile of $B$ decreases from $Q_{0.05}$ to $Q_{0.95}$. Symmetrically, a negative correlation develops as the quantile of $B$ increases from $Q_{0.05}$ to $Q_{0.95}$. As $Q_1$ tends to $Q_{0.0}$ or to $Q_{1.0}$ these correlations tend to be more pronounced. One interpretation is that, for low values of the stability margins, there is a larger uncertainty about the correlation between $B$ and the stability margins.

Correlations among the four analytical determined ratios (A–C in Table 1 and D in equation (8)) plus the ratio of transient times that we determine numerically (E) are shown in Figure 6. It can be seen that the ratios A, D and E are directly correlated. This means that systems with high values (i.e. close to 1) for A will also, on average, have high values for D and E (i.e. close to 1). Similarly, the ratios B and C are directly correlated. On the other hand, the values of ratios B and C change from negatively to positively correlated with the other three ratios as the values of these other three ratios increases.

Summary

The numerical method reproduces the qualitative results that are obtained analytically as should be expected. Furthermore, the numerical comparison extends the analytical results by providing quantitative results. For example, overall feedback inhibition decreases the stability margins of the steady state, which was shown quantitatively to be on average a minimal effect, and increases the robustness of the system, which was shown quantitatively to be a highly significant effect. The numerical approach also provides a way to compare the temporal responsiveness of the alternative models following perturbations in the steady-state concentrations. For our model systems we found that overall feedback inhibition significantly decreases the response time of the reference system (with overall feedback), compared to that of the alternative system (without overall feedback). Finally, we determined how the different ratios are correlated with systemic properties and with parameters of interest, and we presented a way to determine the confidence one should place on these correlations. Thus, the numerical approach has significantly extended the scope of application beyond that of the analytical approach.

Discussion

The method of mathematically controlled comparison has been used since the early 1970s as a powerful tool to characterize alternative designs for several classes of biological systems. In each case, this comparative technique has provided insight into the natural selection of the various designs. The results obtained in some cases are independent of specific parameter values.
Analytical comparisons in this paper demonstrate that the reference model is more robust and has smaller stability margins than the alternative model. They also show that the flux and concentrations in the reference model are less sensitive to changes in demand for end product. However, analytical comparison cannot give us any qualitative information about the relative transient times of the two models, nor can it tell us anything quantitative about the magnitude of the differences in transient times between the two models.

The method of numerical comparison provides information about the alternative designs that could not have been obtained by exclusive use of analytical comparisons. It shows that the relative differences in parameter sensitivities and transient times between the reference and the alternative models are, on average, much larger than those between stability margins. This implies that differences in stability margins are not very relevant for the selection of overall feedback inhibition. Moreover, this approach shows that more than 99% of all reference models have faster transient responses than the corresponding alternative models. This reinforces the idea that overall feedback
inhibition improves the function of these biosynthetic pathways.

With the method of numerical comparison we also show that, among the five critical ratios that characterize the alternative designs in Figure 1, there are two groups within which the ratios are directly correlated with each other. One group includes the ratios A, D and E; the other includes the ratios B and C. Members of the second group are negatively correlated with members of the first group at low values and positively correlated at high values.

The introduction of contour density of ratios plots (i.e. plots having different moving quantiles for the y-axis) provides a measure for the uncertainty in the correlations between ratios and systemic properties of interest. In most of the cases analyzed in this paper the correlation holds with a 90% confidence interval (i.e. the correlation is always positive, negative or null no matter what quantile is used, as can be seen in Table 2). However, in some cases, such as that in Figure 5, there is more uncertainty about the correlations. Although the nature of the correlations will be model and behavioral-class dependent, there are some properties of these contour plots that are general (see Appendix).

Thus, the method of numerical comparison presented in this paper allows one to quantify, and in some cases to eliminate, the uncertainties associated with the analytical approach to mathematically controlled comparison. This generalization allows one to obtain more information from the comparison. It also allows one to focus the comparison on systems that are considered most appropriate for each design, simply by selecting from randomly generated parameter values ensembles of parameter sets that give rise to systems that are considered appropriate with respect to properties of interest. This provides a means to make the comparison more significant.

Acknowledgements
This work was supported in part by a joint Ph.D. fellowship PRAXIS XXI / BD / 9803 / 96 granted by PRAXIS XXI through Programa Gulbenkian de Doutoramentos em Biologia e Medicina (R.A.), US Public Health Service
Appendix

Consider a contour density of ratios plot of property $P_1$ versus property $P_2$, such as the one presented in Figure 5. If $(P_1)_{Q_k}$ represents the $Q_k$ moving quantile curve for property $P_1$, $(P_1)_{Q_m}$ represents the $Q_m$ moving quantile curve for the same property, $k \leq m$, and the same window size is used to calculate the two curves, then contour density of ratios plots have the following generic properties:

1. For any given value of $(P_2)$ on the x-axis, the curve $(P_1)_{Q_k} \leq (P_1)_{Q_m}$.

2. The shape of the curve for $(P_1)_{Q_r}$, with $k \leq r \leq m$, can only change progressively between the format of the curve $(P_1)_{Q_k}$ and the format of the curve $(P_1)_{Q_m}$.

The proof for the first property comes from the fact that, for the same value of $(P_2)$, $(P_1)_{Q_k}$ and $(P_1)_{Q_m}$ are different quantiles of the same sample. Thus, if $k \leq m$ then $Q_k \leq Q_m$.

The proof of the second property is also very simple. From Property 1 we know that $(P_1)_{Q_k} \leq (P_1)_{Q_m}$. Thus, $(P_1)_{Q_k} \leq (P_1)_{Q_r} \leq (P_1)_{Q_m}$. At each value of $(P_2)$, the maximum number of different quantiles is $W$, which is the window size associated with the total sample of size $S$. Let $Q_i/W$ represent the quantile $i/W$, where $i$ can vary from 1 to $W$. As $1/W \to 0$, such that the ratio $W/S \to constant$, $(P_1)_{Q_i/W} \to (P_1)_{Q(i+1)/W}$. Thus, since $(P_1)_{Q(i-1)/W} \leq (P_1)_{Q_i/W} \leq (P_1)_{Q(i+1)/W}$, the shape of the curves will change progressively from $(P_1)_{Q_{0.0}}$ to $(P_1)_{Q_{1.0}}$.

References


