Infection and Atherosclerosis: Is There an Association?

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The role of infectious agents in the etiology of atherosclerosis has long been implicated. More recently, however, a few epidemiological studies have provided data to dispute the positive association between infection and atherosclerosis. We present a complex system approach using the method of loop analysis to examine the association between infection and atherosclerosis under varied assumptions. We find that both positive and negative associations between infection and atherosclerosis can arise, depending on the study design, the etiological assumptions, and sources and directions of change into the system under study. Finally, we demonstrate a set of conditions under which data can be falsely interpreted as lack of any association between infection and atherosclerosis. The method of loop analysis can be used as an effective guide for designing epidemiological studies before such investigations are undertaken.

1. Introduction

Atherosclerotic cardiovascular disease is the leading cause of mortality in the Western societies. The etiology of atherosclerosis is complex and involves both intrinsic (e.g., cholesterol balance, blood pressure) and environmental factors (e.g., diet, nicotine). Atherosclerosis has long been characterized by inflammation and swelling of the arterial wall.

As early as the 1800s, pathologists had already observed the presence of white blood cells in atherosclerotic lesions suggesting atherosclerosis as an inflammatory disorder. Today, this notion is strongly supported by mounting evidence [1]. Therefore, it was quite reassuring when in 1988 Saikku *et al.* provided serologic evidence that *Chlamydia pneumoniae* (*C. pneumoniae*) may be a potential etiologic agent in atherosclerotic cardiovascular disease due to the inflammatory response of a working innate immune defense to infection [2]. Since then other infectious agents [3–4], and more recently influenza [5–8], have been implicated in atherosclerosis. However, *C. pneumoniae* is the most extensively studied infectious agent for its potential role in atherosclerosis. Today, despite mounting evidence in support of a positive association between infection and atherosclerosis [3–16], there are conflicting data, some of which report in favor of no association [17–20], while others imply a negative association [21] between atherosclerosis and infection.

Recently, Danesh *et al.* conducted a nested case-control prospective cohort study to determine an association between *C. pneumoniae* IgG titres and coronary heart disease. The study design included male subjects, randomly selected from general practice registers in 24 British towns during the years of 1978–1980. The subjects were entered into the British Regional Heart Study [17] and followed for 16 years. Of the 5,661 men who provided blood samples, 496 were selected as cases (defined as those who had either fatal coronary events or nonfatal myocardial infarction (MI) by 1996 and had baseline measurements of IgG serum antibodies to *C. pneumoniae*). A total of 989 subjects who were frequency matched to the cases (by towns of residence and age), were randomly selected as controls (defined as those who had survived to the end of the study without a MI and had baseline *C. pneumoniae* measurements). After the 16 year time course, the authors found no strong association between *C. pneumoniae* IgG titres and coronary heart disease.

In another study, Coles *et al.* investigated the association between atherosclerosis and *C. pneumoniae* with a cross-sectional population study [18]. A sample of 1,034 subjects (near equal split in gender) with sera available for testing *C. pneumoniae* IgG and IgA antibodies were selected from 2,000 randomly selected participants in the 1989 Australian National Heart Foundation Perth Risk Factor Prevalence survey. The authors reported a lack of any association between seropositivity to *C. pneumoniae* and carotid atherosclerosis (quantized by carotid ultrasound analysis).

In a recent study, Jackson *et al.* [20] examined the association between influenza vaccination and a reduction in the risk of recurrent coronary events by studying a cohort of survivors from a first MI during an 11-month period. The authors reported that vaccination against influenza did not reduce the risk of recurrent coronary events in the study cohort,

providing another study conferring no association between atherosclerosis and infection.

More notably, however, is a recent prospective study by Kiechl *et al.* in Bruneck, Italy. The authors set out to test the hypothesis that genetic variants of toll-like receptor 4 (TLR4) are related to the development of atherosclerosis [21]. TLR4 is known to confer differences in the inflammatory response to bacterial lipopolysaccharide (LPS). At the 1990 baseline evaluation, they recruited a random sample of the entire population of Bruneck, stratified by gender and age. A total of 810 subjects were screened for the TLR4 polymorphisms Asp299Gly and Thr399lle. The extent of carotid atherosclerosis was assessed using high-resolution duplex ultrasonography. Subjects with the Asp299Gly TLR4 allele exhibited significantly lower levels of intravascular inflammation and lower risk of carotid atherosclerosis. However, they were found to be more susceptible to several bacterial infections when compared to subjects with the wild type TLR4 allele. The homozygous subjects were at an advantage (compared to heterozygotes) exhibiting less vulnerability to infection. This study presents an instance of a negative association between atherosclerosis and infection, although the authors did not specifically characterize it as such.

Incidentally, in the October 2003 issue of *Science*, Lee *et al.* [22] reported the discovery of yet another gene (PPRδ) that has a regulatory effect on the inflammatory status of macrophages. The deletion of PPRδ from mice foam cells was shown to increase the availability of inflammatory suppressors, thereby reducing atherosclerotic lesion areas by more than 50 percent. The authors did not, however, report on the extent of susceptibility to infection, mainly because their goal was to test the hypothesis that PPRδ controls the inflammatory status of macrophages and thus may be a good target for treating atherosclerosis. Although it is tempting to deduce that susceptibility to infection should be heightened in response to PPRδ-regulated attenuating levels of inflammation, this conclusion remains to be tested and verified.

2. Motivation

Why are there so many conflicting studies?

Epidemiological studies of patterns of association among different components of a biological system (such as association between infection and atherosclerosis) are critical to elucidating the underlying disease dynamics, and vital to devising effective intervention or preventive measures. However, statistical inferences made about patterns of associations can be misleading if the study is not designed in a manner to test such factors. In fact, conflicting data are prevalent in epidemiological studies. When incorrect inference is made about associated patterns, if

the data quality is to be trusted, then either the question has not been posed correctly or the study design requires adjustment.

Suppose we ask the question: Is there an association between smoking and heart disease? The answer should invariably be: This depends on where in the dynamics the change is initiated. If smoking is prevalent in a population, then we may also expect a higher rate of heart disease in that population. However, if a population has a high rate of heart disease, we may not necessarily observe a high rate of smoking, in which case a high incidence of heart disease may be associated with other contributing factors. Hence, the answer to the question may vary depending on the source and direction of change and how that affects the causal relationship in the dynamics.

When posing questions about patterns of correlation between components of a dynamic system, the source and direction of change have to be specified for an accurate evaluation of association. Similarly, the observed patterns of correlation between atherosclerosis and infection depend on: where in the system the change is initiated, number of variables included in the system, the presence or absence of links connecting the variables, and their associated directionality. In this paper, we aim to investigate the question of whether there is an association between infection and atherosclerosis. Using the method of loop analysis [23–30] we show that depending on the study design, the source and direction of change, and patterns of connectivity in the underlying network, the answer can vary. Finally, we argue that adopting such a dynamic view is critical for understanding why there are so many conflicting epidemiological studies, and in particular, why data on association between infection and atherosclerosis are in discord.

3. Methodology

The method of loop analysis is most appropriate for the qualitative study of complex systems when quantitative information is either difficult to obtain or simply unavailable [23–30]. Such systems consist of many interacting components and are constantly perturbed by internal or external impacts. In the absence of numerical information, loop analysis can be used to enhance understanding of the underlying dynamics and elucidate patterns of association and dependencies between system components and the direction of change when the system is perturbed from equilibrium. For a thorough introduction to loop analysis we refer the reader to the appendix.

Consider the system of three variables A (atherosclerosis), N (inflammation), and I (infection), as depicted in Figure 1. Infection stimulates an inflammatory response (the positive link from I to N represented by $\rightarrow$), which in turn subsides the infection (the negative link from N to I represented by $-\!\circ$). Inflammation can induce atherogenecity (the

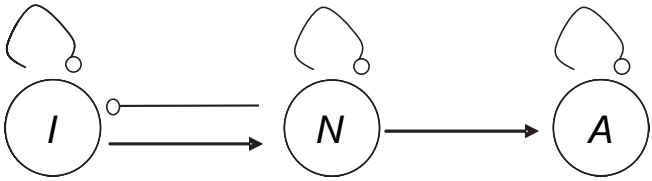

Figure 1. Qualitative model: a system of interactions between atherosclerosis (A), inflammation (N), and infection (I).

positive link from N to A) [31, 32]. It can also be self-damping since it is an expression of chemicals or cells attracted to the infection site from a much larger pool, and because immune components have a finite life span (the negative loop from N to N) [31, 32]. In the absence of intervention, microorganisms may be self-limiting due to competitive exclusion, or crowding (the negative loop from I to I) [33, 34]. Finally in the absence of exogenous factors, atherosclerosis may undergo recovery, thus healing itself and therefore being self-damping (negative loop from A to A). Figure 1 illustrates the $I - N - A$ system.

In order to analyze the feedback system of Figure 1, we need the notion of feedback at level k, F_k (see the appendix). Mathematically we have:

$$F_k = \sum_{m=1}^{k} (-1)^{m+1} L(m, k). \tag{1}$$

Intuitively, equation (1) defines feedback at level k as the net feedback of all the subsystems consisting of k variables in a system of n variables ($k \leq n$). Here $L(m, k)$ is defined as the product of m disjunct loops with k variables ($m \leq k$) where disjunct loops are those loops that have no variable in common (see equation (A.5) in the appendix). For example, the product of two disjunct loops ($m = 2$) with three variables ($k = 3$) would include $A \multimap A$ (the negative loop of size one from A to A) and the negative loop of size two consisting of the link $I \to N$ (I positively regulates N) and the link $N \multimap I$ (N negatively regulates I). In this example, no variables are shared between the two loops. By definition we always have $F_0 = -1$.

For any loop model with n variables there are n points of entry for parameter changes, one for each variable. A table of predictions can then be constructed to show how the growth rate for each variable will change (i.e., whether it increases, decreases, or remains the same), due to the change in its own parameters, or those of other variables. The following formula computes the direction (sign) of change for every such

Effect of parameter change on growth rate

Change in growth rate	I	N	A
I	+	+	+
N	-	+	+
A	?	?	+

Table 1. Association matrix for the patterns of covariation between atherosclerosis (A), inflammation (N), and infection (I). Patterns are summarized by a positive effect (+), negative effect (−), and no association can be made (?).

variable

$$\frac{\partial X_j}{\partial c} = \frac{\sum_{i,k}\left(\frac{\partial f_i}{\partial c}\right) p_{ji} F_{n-k}^{\text{comp}}}{F_n}. \tag{2}$$

Here $\partial X_j/\partial c$ indicates the change in variable X_j with respect to change in parameter c. The formula can be interpreted as follows. Take every variable X_i that includes the parameter c, determine the direction of change caused by c in its own growth rate f_i, that is, whether $\partial f_i/\partial c$ is positive, negative, or zero. Then trace each possible path p_{ji} from X_i to X_j, determine its sign, and multiply every such path by its complementary feedback F_{n-k}^{comp}. Finally, sum over all such variables and paths, and normalize by the overall feedback of the entire system of n variables, F_n (see equation (A.9) in the appendix for derivation). Note that $p_{ii} = 1$, that is, the length of the path from a variable to itself is always the unity. This procedure results in an $n \times n$ table of patterns of covariation among n variables (see Table 1). The table columns indicate the variables being affected by change while the table rows indicate the variables through which the change is initiated. Each entry in the table indicates the qualitative effect (+ or −) of change on each variable growth rate.

4. Results

To compute possible patterns of covariation by equation (2), first we need to compute feedback at level three. By equation (1) we have:

$$\begin{aligned} F_3 &= (-1)^3 L(2,3) + (-1)^4 L(3,3) \\ &= (-1)(A \multimap A)(I \to N)(N \multimap I) + (I \multimap I)(N \multimap N)(A \multimap A) \\ &= (-) + (-) = (-). \end{aligned}$$

Note that $L(1,3) = 0$ since there is no loop consisting of all three variables. For the system to be stable, we must have $F_3 < 0$ (for conditions of stability, see equations (A.2) through (A.6) in the appendix). Suppose that the change in the system is initiated through parameter a_I of the variable I and that $\partial f_I/\partial c_I > 0$. Then we have:

$$\frac{\partial N}{\partial c_I} = \frac{(+)(I \to N)F_1^{\text{comp}}}{F_3} = \frac{(+)(+)(-1)^2(A \multimap A)}{(-)} = (+)$$
$$\frac{\partial A}{\partial c_I} = \frac{(+)(I \to N)(N \to A)F_0^{\text{comp}}}{F_3} = \frac{(+)(+)(+)(-)}{(-)} = (+)$$
$$\frac{\partial I}{\partial c_I} = \frac{(+)F_2^{\text{comp}}}{F_3} = \frac{(+)(-1)^3(N \multimap N)(A \multimap A)}{(-)} = \frac{(+)(-)(-)(-)}{(-)} = (+).$$

This implies that all three variables change in the same direction. In this case, we get a positive association between infection and atherosclerosis (see the first row in Table 1).

Now suppose that the parameter being changed is a_N of variable N (inflammation). Suppose further that $\partial f_N/\partial c_N > 0$. If we apply equation (2) to the three variables A, I, and N we get:

$$\frac{\partial N}{\partial c_N} = \frac{(+)F_2^{\text{comp}}}{F_3} = \frac{(+)(-1)^3(I \multimap I)(A \multimap A)}{(-)} = \frac{(+)(-)(-)(-)}{(-)} = (+)$$
$$\frac{\partial A}{\partial c_N} = \frac{(+)(N \to A)F_1^{\text{comp}}}{F_3} = \frac{(+)(+)(-1)^2(I \multimap I)}{(-)} = \frac{(+)(-)}{(-)} = (+)$$
$$\frac{\partial I}{\partial c_N} = \frac{(+)(-NI)F_1^{\text{comp}}}{F_3} = \frac{(+)(-)(-1)^2(A \multimap A)}{(-)} = \frac{(+)(-)(+)(-)}{(-)} = (-).$$

Thus if the change in the system is initiated through a parameter of inflammation, then inflammation and atherosclerosis change in the same direction while inflammation and infection change in opposite directions (see the second row in Table 1).

By symmetry, if $\partial f_N/\partial c_N < 0$, that is, if the change in parameter a_N causes the growth rate for inflammation to decrease then we have

$$\frac{\partial N}{\partial c_N} = \frac{(-)F_2^{\text{comp}}}{F_3} = \frac{(-)(-1)^3(I \multimap I)(A \multimap A)}{(-)} = \frac{(-)(-)(-)(-)}{(-)} = (-)$$
$$\frac{\partial A}{\partial c_N} = \frac{(-)(N \to A)F_1^{\text{comp}}}{F_3} = \frac{(-)(+)(-1)^2(I \multimap I)}{(-)} = \frac{(-)(-)}{(-)} = (-)$$
$$\frac{\partial I}{\partial c_N} = \frac{(-)(N \multimap I)F_1^{\text{comp}}}{F_3} = \frac{(-)(-)(-1)^2(A \multimap A)}{(-)} = \frac{(-)(-)(+)(-)}{(-)} = (+).$$

Indeed, this is the case in [14]. If the change is initiated through the expression of the TLR4 variant Asp299Gly causing attenuated levels of inflammation, then reduction in inflammation reduces susceptibility to

atherosclerosis while increasing susceptibility to infection. This result can also be interpreted as a negative association between infection and atherosclerosis. Hence adopting a dynamic view of mechanisms of interaction in the $I - N - A$ system can result in two different yet both valid answers to the same question.

Under what condition(s) may there be no association between atherosclerosis and infection? There is more than one way that this can occur. Suppose the change in the system is initiated through a parameter of atherosclerosis and that $\partial f_A/\partial c_A > 0$. In this case we have:

$$\begin{aligned}\frac{\partial A}{\partial c_A} &= \frac{(+)F_2^{\text{comp}}}{F_3} = \frac{(+)[(-1)^2(N \multimap I)(I \to N) + (-1)^3(I \multimap I)(N \multimap N)]}{(-)} \\ &= \frac{(+)[(-) + (-)]}{(-)} \\ &= (+).\end{aligned}$$

But since there is no direct link from A to either I or N, if the change is initiated through one of the parameters of A, the direction of change in I and N cannot be determined under the model of Figure 1, that is, $\partial I/\partial c_A$ = ? and $\partial N/\partial c_A$ = ? (see the third row in Table 1). In other words, under the model in Figure 1, if the parameter change is introduced through atherosclerosis, it is not clear whether there is any association between infection and atherosclerosis. Such uncertainty can be falsely interpreted as lack of association when in fact there is not enough information to draw any conclusive assertion. Table 1 summarizes these results.

Another condition that may incorrectly be interpreted as "no association" between atherosclerosis and infection is when there is more than one point of entry for change in the system. Suppose the change in the system is initiated through parameters of both variables I and N. Suppose further that $\partial f_N/\partial c_N < 0$ due to the expression of the TLR4 variant and $\partial f_I/\partial c_I < 0$ due to (influenza) vaccination. Figure 2 illustrates si-

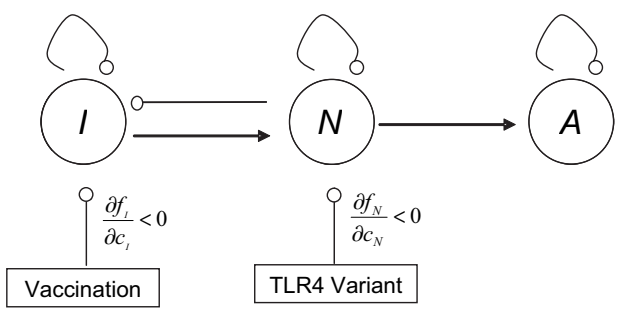

Figure 2. A system of interactions between atherosclerosis (A), inflammation (N), and infection (I) modeling simultaneous parameter changes in N and I.

multaneous parameter changes in I and N in the system of Figure 1. Then we have:

$$\begin{aligned}\frac{\partial I}{(\partial c_N, \partial c_I)} &= \frac{(-)(N \multimap I)F_1^{\text{comp}} + (-)F_2^{\text{comp}}}{F_3} \\ &= \frac{(+) + (-)}{(-)} \\ &= (-) + (+) \\ &= ?\,.\end{aligned}$$

At the same time we have:

$$\begin{aligned}\frac{\partial A}{(\partial c_N, \partial c_I)} &= \frac{(-)(N \to A)F_1^{\text{comp}} + (-)(I \to N)(N \to A)F_0^{\text{comp}}}{F_3} \\ &= \frac{(+) + (+)}{(-)} \\ &= (-) + (-) \\ &= (-).\end{aligned}$$

Then what we observe is a reduction in the rate of atherosclerosis while the direction of change in infection cannot be determined, which can be misinterpreted as lack of association. Indeed, this is the case with the cited articles reporting lack of (strong) association between atherosclerosis and infection [17–20]. These are examples of studies that are not designed in a manner to provide conclusive assessment of such an association because the underlying assumptions about the etiology and direction of change have not been carefully considered. When there is inadequate information to assess the association between two components, the study often would have to be redesigned in a manner that the source(s) of change and the interconnectivity between the components are well specified.

5. Discussion

We proposed a qualitative approach based on the method of loop analysis to investigate why there are so many conflicting data on association between infection and atherosclerosis. We then demonstrated that both a positive and negative association can arise depending on parameters of the study design which encompass the number of variables included in the study, the patterns of interconnectivity between variables, and sources and directions of change that affect the dynamics of etiology in the system under study. Finally, we showed when the study design does not elucidate the sources of change, nor sufficiently captures the underlying interconnectivity in the system, the observed outcomes can

be falsely interpreted as lack of any association between infection and atherosclerosis.

Neither Danesh *et al.* and Coles *et al.* with respect to *C. pneumoniae* [17, 18], nor Jackson *et al.* with respect to influenza [20], found any appreciable association between atherosclerosis and infection. As was demonstrated in our case study of Figure 1 using the method of loop analysis, if the source(s) and direction(s) of change are not clearly specified and assumptions about the etiology of the dynamics are not carefully considered, then at an arbitrary point in time, it may not be possible to establish an association between infection and atherosclerosis.

One possible scenario leading to the false conclusion of lack of any association in a study design is as follows. Suppose some of the cases have high levels of antibody to an infectious agent at baseline due to a TLR4 polymorphism that attenuates inflammation but such a relationship is not considered in the study. Suppose further that the rest of the cases have wild type TLR4 so that high susceptibility to infection also implies high susceptibility to atherosclerosis, yet again not considered in the study. Then what we observe is low incidence of atherosclerosis in the first group since low levels of inflammation implies low atherogenecity, and high incidence of atherosclerosis in the second group. Under this situation, the association between infection and atherosclerosis appears ambiguous and can erroneously be interpreted as lack of any association, when in fact more information should be collected and further investigation is required. Unless the source and direction of change are well identified, and the assumptions about the underlying patterns of interaction between the system components are well characterized, the study design should be considered incomplete. Consequently, any conclusions drawn about the presence or absence of association between atherosclerosis and infection should be considered at best arbitrary.

It is important to note some of the constraints as well as the utility of the presented approach. First, the method of loop analysis does not allow one to assess degrees or strengths of association between variables. As such, a strong association (positive or negative) cannot be distinguished from a weak one, even though such a gradient might be necessary when evaluating the strengths of cause-and-effect relationships (e.g., for devising therapeutic regimens). However, qualitative models such as the method of loop analysis are most appropriate for understanding the underlying dynamics when numerical assessment of the state of variables and parameter values is difficult or impossible. Furthermore, the method has practical implications for designing studies. By considering more than one model for the study investigators can examine the qualitative effects of different variables and patterns of connectivity and choose the design that makes most sense biologically before initiating the study or attempting to collect quantitative infor-

mation about some of the biological parameters, which is often a costly and time consuming task if not intractable.

Second, in loop analysis the underlying assumption is that the system under study is near or at equilibrium, and the subsequent analyses are directed at capturing the new equilibrium values qualitatively when the system is perturbed from its current state of equilibrium. Clearly, such an assumption may be at odds with the state of reality in biological systems. However, as with all mathematical models, loop analysis is not an endeavor to embody an exact presentation of the real world. It is an attempt to simplify a real-world phenomenon in order to capture and understand those aspects of the system that are important to the investigator so that selected critical questions about the dynamics can be answered.

Finally, it should be noted that the system of Figure 1 analyzed here is quite simple. The underlying simplicity, however, is critical for illustrating the idea. Complexity can rapidly increase as more variables and new links are introduced into the system. As such, a well designed study and well planned data collection scheme is evermore important to avoid costly studies with false assertions. A complex system approach such as the method of loop analysis can be used in conjunction with standard design procedures as an effective guide for designing epidemiological studies before such immense investigations are undertaken.

Appendix

A. Introduction to loop analysis

The method of loop analysis is most appropriate for the qualitative study of complex systems when quantitative information is either difficult to obtain or simply unavailable [23–30]. Such systems consist of many interacting components and are constantly perturbed by internal or external impacts. In the absence of numerical information, loop analysis can be used to enhance understanding of the underlying dynamics and elucidate patterns of association and dependencies between system components that may not be obvious.

There is a one-to-one correspondence between loop models and systems of differential equations, where a system of n differential equations represents a community of n interacting components or variables. These variables often represent component abundances or growth rates. Let X_i be the ith variable. Then we define the rate of change or the growth rate of X_i as follows:

$$\frac{dX_i}{dt} = f_i(X_1, X_2, \ldots X_n; C_1, C_2, \ldots) \tag{A.1}$$

where C_b represents a potential parameter of the system, such as biolog-

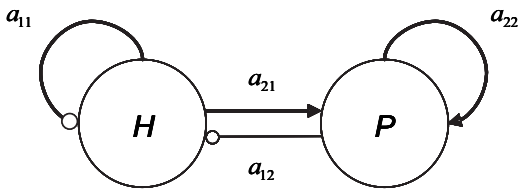

Figure A.1. A predator–prey system with a self-damped herbivore (H), and a self-accelerating predator (P).

ical properties of the variables. If we assume that a system (community) is at or near equilibrium, we can then examine its local stability properties when $dX_i/dt = 0$, for $i = 1, \ldots, n$.

Loop models are also signed digraphs, constructed from the structure of the interaction matrix, or the so-called community matrix in ecology, of coefficients of the X_i evaluated at equilibrium [25]. Given a community matrix **A** as follows:

$$\mathbf{A} = \begin{pmatrix} \frac{\partial f_1}{\partial X_1} & \frac{\partial f_1}{\partial X_2} & \cdots & \frac{\partial f_1}{\partial X_n} \\ \vdots & \vdots & \vdots & \vdots \\ \frac{\partial f_n}{\partial X_1} & \frac{\partial f_n}{\partial X_2} & \cdots & \frac{\partial f_n}{\partial X_n} \end{pmatrix} = \begin{pmatrix} a_{11} & a_{12} & \cdots & a_{1n} \\ a_{21} & a_{22} & \vdots & a_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ a_{n1} & a_{n2} & \cdots & a_{nn} \end{pmatrix}$$

where a_{ij} is the coefficient of X_j in f_j, that is, the effect of change in variable X_j on the growth rate of variable X_i, one can translate the community matrix **A** uniquely into a signed digraph. The variables of the system are the vertices of the graph, and the coefficients of the community matrix are the edges or links of the graph.

The coefficients a_{ij} of the community matrix are readily taken and translate into the effect of the variable X_j on the growth rate of the variable X_i. If the sign of a_{ij} is negative, there will be a negative link from X_j to X_i, represented by the symbol $-\!\circ$. If the sign of a_{ij} is positive in the original matrix, meaning X_j has a positive impact on the growth rate of variable X_i, there will be a positive link from X_j to X_i, represented by the symbol $\rightarrow$.

In a signed digraph there are two types of loops: conjunct and disjunct. Conjunct loops consist of those loops that have at least one variable in common. Disjunct loops have no variable in common. Figure A.1 provides an illustration of loop models for a predator–prey system, where H stands for herbivore and P for predator.

A.1 Conditions for stability

The stability properties and the local behavior of systems of differential equations in the neighborhood of their critical points have been well

studied. Here, we relate the general conditions of stability, as understood in systems theory, to the notion of feedback of a system.

The characteristic polynomial $p(\lambda)$ of a community matrix $\mathbf{A}$ is defined as $|\mathbf{A} - \lambda\mathbf{I}|$, where $\mathbf{I}$ is the identity matrix. For instance, the characteristic polynomial of a 3×3 matrix is:

$$p(\lambda) = \begin{pmatrix} a_{11} - \lambda & a_{12} & a_{13} \\ a_{21} & a_{22} - \lambda & a_{23} \\ a_{31} & a_{32} & a_{33} - \lambda \end{pmatrix}.$$

Expanding the determinant we get:

$$\begin{aligned} p(\lambda) = {} & \lambda^3 - [a_{11} + a_{22} + a_{33}]\lambda^2 \\ & + [(a_{11}a_{22} - a_{12}a_{21}) + (a_{11}a_{33} - a_{13}a_{31}) + (a_{22}a_{33} - a_{23}a_{32})]\lambda \\ & - [(a_{11}(a_{22}a_{33} - a_{23}a_{32}) - a_{12}(a_{23}a_{31} - a_{21}a_{33}) \\ & \quad + a_{13}(a_{21}a_{32} - a_{22}a_{31})] \end{aligned} \tag{A.2}$$

this can be generalized for an n-dimensional matrix as:

$$p(\lambda) = \lambda^n + \sum_{k=1}^{n-1} (-1)^k D_k \lambda^{n-k} \tag{A.3}$$

where D_k is the sum of all principal determinants of order k, corresponding to subsystems of k variables. But every such D_k can also be written as a sum of products of disjunct loops as can be investigated from equation (A.2). That is, we have:

$$D_k = \sum_{m=1}^{k} (-1)^{k-m} L(m, k) \tag{A.4}$$

where $L(m, k)$ is defined as the product of m disjunct loops with k variables. Note that for an $n \times n$ matrix, D_n is the determinant of the square matrix. We then transform this value into the measure of the feedback of a matrix. Specifically, we define the notion of "feedback at level k" as follows:

$$\begin{aligned} F_k &= (-1)^{k+1} D_k \qquad \text{for } k = 1 \ldots n \\ &= (-1)^{k+1} \sum_{m=1}^{k} (-1)^{k-m} L(m, k) \qquad \text{by equation (A.4)} \\ &= \sum_{m=1}^{k} (-1)(-1)^{2k-m} L(m, k) \\ &= \sum_{m=1}^{k} (-1)^{m+1} L(m, k) \qquad \text{since } (-1)^{2k-m} = (-1)^{-m} = (-1)^m \end{aligned} \tag{A.5}$$

where feedback at level 0 is defined as $F_0 = -1$.

In this way, feedback at level k is the net feedback of all the subsystems of k variables in a system of n variables, where $k = 1 \ldots n$.

As an example for the calculation of feedback terms, consider the predator–prey system of Figure A.1. At level 1, $F_1 = -a_{11} + a_{22}$; and at level 2,

$$F_2 = (-1)^2(-a_{12})(a_{21}) + (-1)^3(-a_{11})(a_{22}) = -a_{12}a_{21} + a_{11}a_{22}.$$

Equipped with our definition of feedback at level k, as in equation (A.5), we can rewrite equation (A.3) as:

$$\begin{aligned} p(\lambda) &= \lambda^n + \sum_{k=1}^{n-1}(-1)^k(-1)^{k-m}L(m,k)\lambda^{n-k} \\ &= \lambda^n - \sum_{k=1}^{n-1} F_k\lambda^{n-k} \qquad \text{by equation (A.5).} \end{aligned} \tag{A.6}$$

The characteristic polynomial resulting from equation (A.6) involves the feedback terms as coefficients. Although, one may not be able to generally solve polynomials of higher orders, one can determine the sign of $\mathrm{Re}(\lambda_i)$ using the coefficients of such polynomials. By the Routh–Hurwitz theorem, for the system to be locally stable, that is, for $\mathrm{Re}(\lambda_i) < 0$, for $i = 1 \ldots n$, the coefficients of the polynomial must satisfy two conditions [25, 28, 29, 35]:

1. $F_k < 0, \forall k$.
2. Alternate Hurwitz determinants up to order n must be positive.

If the roots of the polynomial are real, then condition 1 alone suffices and guarantees that all the roots are negative, and hence the system is stable. But if some or all of the roots are complex, then conditions 1 and 2 together imply that the system is oscillatory, but returns to equilibrium through damped oscillations, and hence stable. The second condition needs more elaboration. A Hurwitz determinant of order k, H_k, is defined as follows:

$$H_k = \begin{vmatrix} -F_1 & -F_3 & -F_5 & \cdots & -F_{2k-1} \\ -F_0 & -F_2 & -F_4 & \cdots & -F_{2k-2} \\ 0 & -F_1 & -F_3 & \cdots & -F_{2k-3} \\ 0 & -F_0 & -F_2 & \cdots & -F_{2k-4} \\ 0 & 0 & -F_0 & \cdots & -F_{2k-5} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & -F_k \end{vmatrix}.$$

For instance, in a system of three variables, where only the sign of H_2 needs to be checked, $H_2 = F_1F_2 + F_3 > 0$ implies that negative feedback coming from shorter loops, in this case levels 1 and 2 combined, must

be stronger than the negative feedback coming from the longer loops, in this case feedback at level 3. Since, in equation (A.6), lower order feedbacks are paired with the higher orders of λ, intuitively this means that if λ is small, we get slow damping, and fast damping if λ is large [25, 29].

A.2 The effect of change on the abundance of variables

What is the impact of parameter change in one variable on the abundance, or growth rate, of other variables in the system?

Suppose the change in the system is initiated through a parameter C_h of one or more variables (see equation (A.1)). To determine the effect of change on the equilibrium abundance of system components, we differentiate equation (A.1) with respect to C_h for each variable in the system and set it equal to zero to get:

$$\sum_{j=1}^{n}\left(\frac{\partial f_i}{\partial X_j}\right)\left(\frac{\partial X_j}{\partial C_h}\right)+\left(\frac{\partial f_i}{\partial C_h}\right)=0, \qquad i=1\ldots n \tag{A.7}$$

which can be represented in matrix notation as:

$$\begin{pmatrix} a_{11} & a_{12} & \cdots & a_{1n} \\ a_{21} & a_{22} & \vdots & a_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ a_{n1} & a_{n2} & \cdots & a_{nn} \end{pmatrix}\begin{pmatrix} \dfrac{\partial X_1}{\partial C_h} \\ \dfrac{\partial X_2}{\partial C_h} \\ \vdots \\ \dfrac{\partial X_n}{\partial C_h} \end{pmatrix}=\begin{pmatrix} -\dfrac{\partial f_1}{\partial C_h} \\ -\dfrac{\partial f_2}{\partial C_h} \\ \vdots \\ -\dfrac{\partial f_n}{\partial C_h} \end{pmatrix}.$$

By algebra, we can solve for $\partial X_j/\partial C_h$, $j=1\ldots n$, as follows:

$$\frac{\partial X_j}{\partial C_h}=\frac{\begin{vmatrix} a_{11} & a_{12} & \cdots & -\frac{\partial f_1}{\partial C_h} & \cdots & a_{1n} \\ a_{21} & a_{22} & \cdots & -\frac{\partial f_2}{\partial C_h} & \cdots & a_{2n} \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ a_{n1} & a_{n2} & \cdots & -\frac{\partial f_n}{\partial C_h} & \cdots & a_{nn} \end{vmatrix}}{\begin{vmatrix} a_{11} & a_{12} & \cdots & a_{1j} & \cdots & a_{1n} \\ a_{21} & a_{22} & \cdots & a_{2j} & \cdots & a_{2n} \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ a_{n1} & a_{n2} & \cdots & a_{nj} & \cdots & a_{nn} \end{vmatrix}}. \tag{A.8}$$

The intuition behind equation (A.8) is that its denominator is simply the feedback at level n, that is, F_n. The numerator needs more elaboration before turning it into feedback notation. Note that the effect of substituting the jth column of the community matrix with $-\partial f_j/\partial C_h$, $j=1\ldots n$, is to break all the closed loops that have the link a_{ij} in common. The

numerator is the sum of all such $-\partial f_j/\partial C_h$, $j = 1 \ldots n$, each accompanied by a coefficient. The coefficient of $-\partial f_j/\partial C_h$ is the sum of all possible products of open paths between X_i and X_j, each multiplied by all the closed loops that share no variables with the open path considered. But this simply means that each open path is multiplied by the net feedback of the complement subsystem. Now, let $P_{ji}^{(k)}$ represent the open path from X_i to X_j consisting of k variables, and F_{n-k}^{comp} to represent the feedback of the complement subsystem of size $n-k$, that is, the subsystem remaining after excluding the open path of k variables from the system. Then, equation (A.8) can be rewritten in terms of feedback loops as follows [25, 29]:

$$\frac{\partial X_j}{\partial C_h} = \frac{\sum_{i,k} \left(\frac{\partial f_j}{\partial C_h}\right) P_{ji}^{(k)} F_{n-k}^{\text{comp}}}{F_n} \qquad j = 1 \ldots n. \tag{A.9}$$

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