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MULTISTABILITY, OSCILLATIONS AND BIFURCATIONS IN FEEDBACK LOOPS

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Dedicated to Horst R. Thieme on the Occasion of his 60th Birthday

Abstract. Feedback loops are found to be important network structures in regulatory networks of biological signaling systems because they are responsible for maintaining normal cellular activity. Recently, the functions of feedback loops have received extensive attention. The existing results in the literature mainly focus on verifying that negative feedback loops are responsible for oscillations, positive feedback loops for multistability, and coupled feedback loops for the combined dynamics observed in their individual loops. In this work, we develop a general framework for studying systematically functions of feedback loops networks. We investigate the general dynamics of all networks with one to three nodes and one to two feedback loops. Interestingly, our results are consistent with Thomas' conjectures although we assume each node in the network undergoes a decay, which corresponds to a negative loop in Thomas' setting. Besides studying how network structures influence dynamics at the linear level, we explore the possibility of network structures having impact on the nonlinear dynamical behavior by using Lyapunov-Schmidt reduction and singularity theory.

1. **Introduction.** Recently, the role of feedback loop networks has received extensive attention [2, 7, 8, 24, 27, 30] and it has become accepted that the concept of feedback may be useful as a framework for understanding how intracellular signaling systems elicit specific cell behavior [6]. A feedback loop is a cycle in a directed graph whose edges can represent either positive or negative inputs. In a biological network, the nodes can represent concentrations of proteins or genes. In this context, a positive (resp. negative) edge indicates that an increasing of the concentration of the tail node will lead to an increasing (resp. decreasing) of the production rate of the head node. A positive feedback loop consists of an even number of negative inputs while a negative feedback loop consists of an odd number of negative inputs.

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Feedback loop motifs are important to maintain normal biological activities [3, 9, 10, 19, 26]. For instance, it has been shown that circadian rhythm, which is associated with many organisms' daily activities such as sleep-wake cycle, is generated by loops of genes that communicate through positive and negative feedback [10, 26]. Feedback loops also play significant roles in developmental networks that govern the fates of cells during the transition of an egg into a multi-cellular organism [9]. For example, a two-node positive feedback loop usually has bi-stability which allows cells to make irreversible decision. Positive feedback loops are widely viewed to be responsible for multistability. It was actually conjectured by Thomas [31] and proved by Soulé [28] that the presence of positive feedback loop is a necessary condition for multistibility. Negative feedback loops are considered to be responsible for oscillations [7, 8, 31]. For example, after stresses such as DNA damage, the concentration of the tumor suppressor p53 exhibits oscillatory ups and downs. This oscillating behavior is due to the negative feedback loop consisting of p53 and its negative regulator Mdm2 [19]. In biological networks, feedback loop motifs often occur as a coupled structure rather than a single isolated form [17, 18]. Therefore, it is important to understand the dynamics of coupled feedback loops. To our knowledge, the current existing literature about the role of coupled feedback loops focuses on verifying that they have the combinatorial dynamics of the individual loops that constitute the coupled structure. However, coupled systems may have dynamics that cannot be predicted from their individual components.

In this work, we develop a framework that enables a systematic study of the functions of feedback loop networks, which we will describe in Section 2. Under this framework, we consider feedback loops with one to three nodes and one to two loops, whose architecture is given in Figure 1. We focus on these feedback loop networks because networks with up to three nodes are small enough to analyze in a theoretical framework, but large enough to admit complex dynamical behavior as shown in several works [20]. Since multistability and oscillations are very common and play important roles in cellular systems [1, 19, 22, 25, 26], we investigate mainly the existence of these two properties in the networks listed in Figure 1. We find that the presence of negative feedback loop is essential for the networks in Figure 1 to admit oscillations even though we model the dynamics of each node in the network by a decay term. In Thomas [31] setting, this assumption is equivalent to have a negative feedback loop. Consequently, our result suggest that oscillations in feedback loops are not determined by negative loops alone: some extra network structure might be needed to support oscillations.

In general, multistability occurs via saddle-node bifurcation. However, it might be possible for some networks to exhibit degenerate bifurcations, such as transcritical and pitchfork type, due to the presence of certain symmetries [11, 20]. For example, if one type of transcription factors regulates different genes in a similar way, the associated regulatory network have some local 'symmetry'. Therefore, we explore whether or not network architecture of feedback loops forces degenerate codimension-one steady-state bifurcations to occur. We investigate this possibility for networks in family No. 5 because they have more symmetry from network architecture perspective.

The paper is constructed as follows. In Section 2, we introduce the framework for the study of the dynamical behavior of feedback loop networks listed in Figure 1. In Section 3, we first study which networks admit multistability and oscillations, then

we classify codimension-one steady-state bifurcations for the networks of family No. 5. The main results are discussed in Section 4.

2. **Feedback loops and admissible vector fields.** There are two types of inputs in feedback loops: positive (excitatory) and negative (inhibitory) inputs. We use the conventional way to represent these two types of inputs: $\dashv$ representing repression, $\rightarrow$ representing activation. We only consider feedback loops and their coupled networks with one to three nodes and one to two loops. All such possible networks, up to permutation of cells, have one of the network forms listed in Figure 1. An exhaustive classification of networks with three nodes and more edges is presented by Tyson et al. [32]. Interestingly, all of these networks in Figure 1 have been found in transcriptional regulatory networks [1, 5, 17, 18].

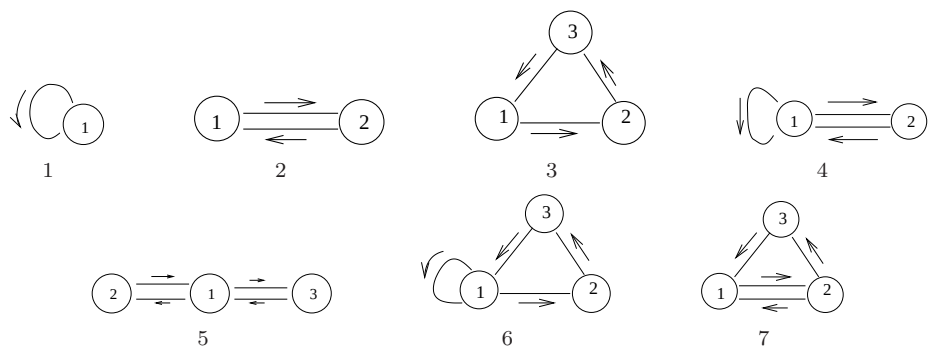


FIGURE 1. The possible network forms of one and two feedback loops with one to three nodes, the arrows beside edges represent the direction of inputs: the head nodes receive inputs from the tail nodes and the inputs can be either positive or negative.

A simple mathematical formalism of feedback loops in transcriptional networks is suggested by Gardner et al. [12], which is employed by Kim et al. [18]. In this formalism, positive and negative inputs are modeled by Hill type functions: increasing Hill functions if the inputs are positive and decreasing Hill functions if the inputs are negative. The internal dynamics of each node is described by a first-order polynomial, which consists of a linear degradation term and a constant basal synthesis term. An example of a model constructed by Kim et al. [18] that uses this ideas is given in (1). The associated network belongs to family No. 2 listed in Figure 1. The system (1) models the interactions between two biochemical species: X activates the transcription of the gene of Y and Y represses the transcription of the gene of X [18].

$$\begin{aligned} \dot{Y} &= -K_{dy}Y + K_{by} + \frac{V_x(X/K_{xy})^H}{1+(X/K_{xy})^H} \\ \dot{X} &= -K_{dx}X + K_{bx} + \frac{V_y}{1+(Y/K_{yx})^H}, \end{aligned} \tag{1}$$

where V_*, H, and K_* are parameters.

Motivated by the model strategy described above, we define the *admissible vector fields* of feedback loops in the context of transcriptional regulatory networks as follows.

Let $\overline{\mathbf{R}^+} = \mathbf{R}^+ \cup \{0\}$. The *admissible vector fields* of a feedback loop satisfy

1. the state variable of each node is one dimensional.
2. the internal dynamics of each node has the form of $-h(x)$, where $h : \overline{\mathbf{R}^+} \to \overline{\mathbf{R}^+}$ and $h(x)$ is a strictly increasing function.
3. each positive input is represented by a strictly monotonically increasing function with domain and range in $\overline{\mathbf{R}^+}$; each negative input is represented by a strictly monotonically decreasing function with domain and range in $\overline{\mathbf{R}^+}$.

For example, the admissible vector field of the negative feedback loop belonging to family No. 2 in Figure 1 has the form

$$\begin{aligned} \dot{x}_1 &= -h^1(x_1) + f^1(x_2) \\ \dot{x}_2 &= -h^2(x_2) + f^2(x_1), \end{aligned}$$

where $x_i \geq 0$, $f^i(x_j) \geq 0$, and $h^i(x_i) \geq 0$. Also, we assume that $h^i(x_i)$ are strictly monotonically increasing.

3. Dynamics of the networks.

In this section, we first examine networks in Figure 1 for their capacity of exhibiting multistability and oscillation. Then, we study possible codimension-one steady-state bifurcations for the family of networks No. 5.

3.1. Multistability and oscillation.

Craciun and his collaborators [4, 23] have recently developed a graph-theoretic method to determine whether multistability and oscillation via Hopf bifurcation can occur in biochemical networks. However, under our specific problem setting, it is much easier to compute directly equilibria and Hopf bifurcation conditions. Next we illustrate how we determine those conditions using a network in family No. 3. We consider the admissible vector fields associated to networks No. 3 given by

$$\begin{aligned} \dot{x}_1 &= -h^1(x_1) + f^1(x_3) \\ \dot{x}_2 &= -h^2(x_2) + f^2(x_1) \\ \dot{x}_3 &= -h^3(x_3) + f^3(x_2), \end{aligned} \tag{2}$$

In addition, we assume that except for f^3 being monotonically decreasing in $\overline{\mathbf{R}^+}$, all other functions in (2) are monotonically increasing.

By letting the right-hand sides of (2) to be equal to zero, we obtain

$$x_3 = ((h^3)^{-1} \circ f^3)(x_2), \quad x_1 = ((f^2)^{-1} \circ h^2)(x_2), \quad h^1(x_1) = f^1(x_3). \tag{3}$$

Then, substitution of the first two equations in (3) into the last one yields

$$(h^1 \circ (f^2)^{-1} \circ h^2)(x_2) = (f^1 \circ (h^3)^{-1} \circ f^3)(x_2).$$

Note that the composition of two increasing (decreasing) functions is increasing while the composition of an increasing and a decreasing function is decreasing. Hence, $(h^1 \circ (f^2)^{-1} \circ h^2)(x_2)$ is monotonically increasing and $(f^1 \circ (h^3)^{-1} \circ f^3)(x_2)$ is monotonically decreasing. Since there is at most one intersection point of graphs of an increasing function and a decreasing function, there is *at most one equilibrium* for the negative feedback loop in family No. 3 given in Figure 2.

Next we consider the possibility that Hopf bifurcation exists in system (2). That is, we check whether or not the associated Jacobian admits a pair of pure imaginary eigenvalues.

The Jacobian matrix of (2) is

$$J = \begin{pmatrix} -h_1^1 & 0 & f_3^1 \\ f_1^2 & -h_2^2 & 0 \\ 0 & f_2^3 & -h_3^3 \end{pmatrix},$$

where $h_j^i = \frac{\partial h^i}{\partial x_j}$ and $f_j^i = \frac{\partial f^i}{\partial x_j}$. The associate characteristic equation is

$$p(\lambda) = \lambda^3 + a_1\lambda^2 + a_2\lambda + a_3 = 0,$$

with

$$\begin{aligned} a_1 &= h_1^1 + h_2^2 + h_3^3, \\ a_2 &= h_1^1 h_2^2 + h_2^2 h_3^3 + h_1^1 h_3^3 \\ a_3 &= h_1^1 h_2^2 h_3^3 - f_3^1 f_1^2 f_2^3. \end{aligned}$$

Hence, the Hurwitz determinant is given by

$$H_2 = a_1 a_2 - a_3 = (h_1^1 h_2^2 + h_1^1 h_3^3) + (h_2^2 + h_3^3) h_2^2 h_3^3 + f_3^1 f_1^2 f_2^3.$$

By Lienard-Chipart criterion [21], equation (3.1) has a pair of pure imaginary eigenvalues if and only if $a_2 > 0$ and $a_1 a_2 - a_3 = 0$. The coefficient $a_2 > 0$ since all h^i are increasing functions and therefore $h_j^i > 0$. Also, except for the last term $f_3^1 f_1^2 f_2^3$, all other terms in the expression of $a_1 a_2 - a_3$ are positive. Hence, $a_1 a_2 - a_3 = 0$ is possible only when $f_3^1 f_1^2 f_2^3 < 0$. Consequently, the feedback loop of networks No. 3 in Figure 1 has to be negative. In Figure 2 we present one network in Family No. 3 that admits Hopf bifurcation.

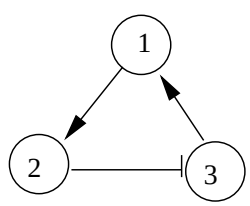


FIGURE 2. A three-node negative feedback loop. This network exhibits oscillations via Hopf bifurcation.

Using the same technique we can study the capacity of multistability and oscillations via Hopf bifurcation for all other networks in Figure 1. The details are given in Appendix A.

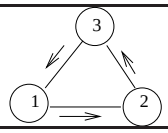
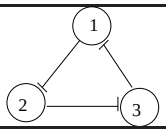
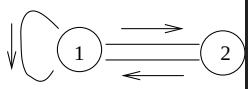
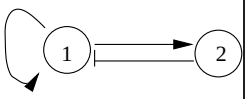
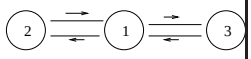
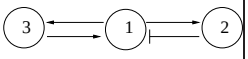
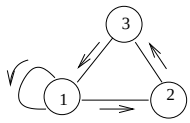
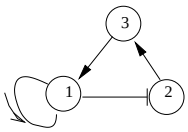
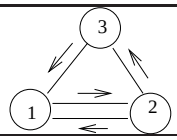
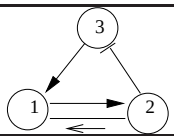
Our results are summarized as follows:

1. The networks with at least one positive feedback loop admit multistability and steady-state bifurcation. Otherwise, the networks have at most one equilibrium. That is, for networks in Figure 1 the presence of positive feedback loops is necessary for multistability.

2. The results concerning the capacity of the networks for Hopf bifurcation are listed in Table 1. One feedback loop with only one or two nodes, that is family No. 1 and No. 2, can not have Hopf bifurcation. However, negative feedback loops with three nodes, that is, family No. 3, admit Hopf bifurcation if their three-node loop is negative. The family No. 4 needs to contain a positive one-cell loop and a negative two-cell loop to admit Hopf bifurcation. Such network

architecture has been actually employed as a model in [6]. The networks of family No. 5 support Hopf bifurcation if one two-node loop is positive and another is negative. The remaining two families have a common property that networks with a three-node negative loop support Hopf-bifurcation.

TABLE 1. The capacity and conditions for admitting Hopf bifurcation. The arrows beside the edges represent directions of inputs, but the inputs can be either positive or negative. The networks in the third column are representative networks that satisfy the conditions on the fourth column.

No.	Networks	Admitting Hopf bifurcation	
1		None	
2		None	
3			negative feedback loops admit Hopf bifurcation.
4			networks with one-node loop being positive and two-node loop being negative admit Hopf bifurcation.
5			networks with one positive feedback loop and one negative feedback loop admit Hopf bifurcation.
6			networks with negative loops or negative three-node loop and weak auto-positive feedback loop admit Hopf bifurcation.
7			networks with negative three-nodes loop admit Hopf bifurcation.

3.2. **Bifurcation analysis.** By the discussion in Section 3.1, it follows that a network in Figure 1 admits steady-state bifurcation if it admits multistability. Multistability often occurs with two stable equilibria separated by an unstable equilibrium. In general setting, such multistability can be obtained via saddle-node bifurcation (see for example [23] and references therein).

Degenerate codimension-one bifurcations, such as pitchfork bifurcations, generically occur only in the presence of some kind of symmetry [14, 16, 20], including localized symmetry as defined in the theory of coupled cell systems developed by Stewart et al. [15, 29]. In the context of this paper, a system where localized

symmetry can be present would be a transcriptional network with one type of transcription factors regulating various genes in a similar way. In this scenario it might be possible for the transcriptional networks to have degenerate dynamics.

In this section we explore if degenerate codimension-one steady-state bifurcations can occur in networks listed in Figure 1. We investigate such possibility only for coupled feedback loops in family No. 5. We choose to study this particular family because those networks have more symmetry, which from our point of view make them a good candidate for having degenerate dynamics. We next classify codimension-one steady-state bifurcations in networks No. 5 by applying Lyapunov-Schmidt reduction technique (see for example [13, Chapter 1]).

The admissible vector fields of the networks have the form

$$\dot{X} = F(X, \lambda), \tag{4}$$

where $\lambda \in \overline{\mathbf{R}^+}$ is a bifurcation parameter, $X = (x_1, x_2, x_3) \in \overline{\mathbf{R}^+}^3$, and

$$\begin{aligned} F(X,\lambda) &= (F^1(X,\lambda), F^2(X,\lambda), F^3(X,\lambda)) \\ &= (-h^1(x_1) + f^1(x_2, x_3, \lambda), -h^2(x_2) + f^2(x_1, \lambda), -h^3(x_3) + f^3(x_1, \lambda)). \end{aligned} \tag{5}$$

We assume that (4)-(5) has an equilibrium $X_0 = (x_1^0, x_2^0, x_3^0)$ when $\lambda = \lambda_0$ and the Jacobian J at the equilibrium has a zero eigenvalue. Generically, the kernel space of the Jacobian is one dimensional. We now investigate what kind of codimension-one steady-state bifurcation can occur.

Using Lyapunov-Schmidt reduction method, we can reduce the original steady-state problem $F(X, \lambda) = 0$ to a one-dimensional algebraic equation (see Appendix B for the detailed calculation). The reduced equation has the form:

$$g(x, \lambda) = 0, \qquad x \in \mathrm{Ker}(J),$$

and its lower order derivatives satisfy:

$$\begin{aligned} g(X_0,\lambda_0) &= 0 \\ g_x(X_0,\lambda_0) &= 0 \\ g_\lambda(X_0,\lambda_0) &= h_2^2 h_3^3(-h_\lambda^1 + f_\lambda^1) + (-h_\lambda^2 + f_\lambda^2) f_2^1 h_3^3 + (-h_\lambda^3 + f_\lambda^3) f_3^1 h_2^2 \\ g_{xx}(X_0,\lambda_0) &= f_{22}^1 (f_1^2)^2 (h_3^3)^3 h_2^2 + f_{33}^1 (f_1^3)^2 (h_2^2)^3 h_3^3 + 2 f_{23}^1 f_1^2 f_1^3 (h_2^2)^2 (h_3^3)^2 \\ &\quad + f_{11}^2 f_2^1 (h_2^2)^2 (h_3^3)^2 + f_{11}^3 f_3^1 (h_2^2)^2 (h_3^3)^2 - h_{11}^1 (h_2^2)^3 (h_3^3)^3 \\ &\quad - h_{22}^2 (f_1^2)^3 (h_3^3)^3 - h_{33}^3 (f_1^3)^3 (h_2^2)^3, \end{aligned} \tag{6}$$

where $h_j^i = \frac{\partial h^i}{\partial x_j}$, $h_\lambda^i = \frac{\partial h^i}{\partial \lambda}$, $f_{jk}^i = \frac{\partial^2 f^i}{\partial x_j \partial x_k}$, and $f_\lambda^i = \frac{\partial f^i}{\partial \lambda}$, with $i, j, k = 1, 2, 3$.

Observe that generically, the right-hand side of the last two equations in (6) are nonzero. Hence, generically $g_\lambda \neq 0$ and $g_{xx} \neq 0$. By bifurcation recognition method [13] it follows that, generically, only saddle-node bifurcation can occur in (4)-(5).

In Remark 1 we explore scenarios in which networks in family No. 5 can exhibit either symmetry or local symmetry and investigate if, in those cases, degenerate bifurcation can occur.

Remark 1. Some networks belonging to the family No. 5 in Table 1 may exhibit symmetry if we choose vector fields among the admissible ones satisfying the following two conditions. One condition is that the dynamics of nodes 2 and 3 are described by identical differential equations. That is, $h^2(x) = h^3(x) \equiv h(x)$, $f^2(x) = f^3(x) \equiv f(x)$. The other condition is that the inputs from nodes 2 and 3 to node 1 are identical, that is, $f^1(x_2, x_3) = f^1(x_3, x_2)$. Under this assumptions the networks have $\mathbf{Z}_2 = \langle (2, 3) \rangle$ symmetry. The presence of $\mathbf{Z}_2$ symmetry typically leads to pitchfork bifurcation [14]. However, that is not the case for the subfamily of

networks No. 5 with symmetry since for those networks g_λ generically is not equal to zero even under the symmetric assumption.

Next we consider a scenario in which some networks in family No. 5 have local symmetry. More precisely, we assume $h^2(x) = h^3(x) \equiv h(x)$, $f^2(x) = f^3(x) \equiv f(x)$, but $f_2^1(x_2, x_3) = -f_3^1(x_3, x_2)$. In addition we assume that dynamics of node 1 do not depend on the bifurcation parameter λ, that is, $f_\lambda^1 = 0 = h_\lambda^1$. Under these assumptions we claim that if $g = 0 = g_x$, then $g_\lambda \neq 0$. The proof is as follows. Let $f' = f_1^2 = f_1^3$ and $h' = h_2^2 = h_3^3$. Observe that the condition $g_x = 0$ implies that $-h_1^1(h')^2 + f'h'(f_2^1 + f_3^1) = 0$. Since $h_1^1(h')^2 > 0$ the quantity $f'h'(f_2^1 + f_3^1)$ has to be positive. This contradicts the assumption $f_2^1 = -f_3^1$. Therefore, g_λ can not be zero.

4. **Discussion.** Thomas [31] conjectured that the presence of a negative loop (circuit) is a necessary condition for stable periodicity. But in the conjecture, he defines the vector field as

$$\dot{X} = F(X),$$

which integrates the degradation term $-h(x_i)$ in F. In this work, we separate the interaction terms from degradation terms. That is, we do not count degradation terms as a negative feedback loops. However, Thomas' conjecture seems still valid in our setting. That may imply negative feedback loop is not the only feature of network architecture responsible for sustained oscillations. That is, some extra structure might be needed for a feedback loop network to support oscillations.

As remarked in bibliography (see for example [33]), our results suggest that in order to be capable of producing oscillatory behavior the one feedback loop networks with two nodes may have evolved using a combination of two strategies: (1) acquiring the ring simplest architecture, which forces the number of nodes to increase from two to three; (2) increase the number of loops in the architecture by forming coupled loops.

Regarding codimension-one steady-state bifurcations from an equilibrium, we choose to study those bifurcations that can occur in networks in the family No. 5 for its particular feature pointed above. We show, analytically, that generically, a saddle-node bifurcation from an equilibrium occurs in the system. The classification of codimension-one steady-state bifurcations for the remaining networks in Table 1 can be performed using the systematic approach we use in this work.

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Appendix A. **Capacity for multistability, steady-state bifurcation and oscillations.** In this section, we present detailed calculations to support the results obtained in Section 3.1. We first introduce some known results employed in the calculations. Then we give the computational details for several representative of networks in Figure 1.

- Multistability is obtained by directly computing algebraic equations. Our conclusions about multistability are based on the following properties on monotonically functions:

  Let f_1 and f_2 be two monotonic functions, then the composition of the two functions satisfies:

1. $f_1 \circ f_2$ is monotonically increasing if both functions are either positive or negative.
2. $f_1 \circ f_2$ is monotonically decreasing if one of them is monotonically decreasing and the other is increasing.

Let $f_1(x)$ and $f_2(x)$ be two monotonic functions, then

1. if one of the function is monotonic decreasing and the other is increasing, then there is at most one solution to the algebraic equation: $f_1(x) = f_2(x)$.
2. if both of the functions are either monotonically decreasing or increasing, then there might be multiple solutions to the equation $f_1(x) = f_2(x)$.

- The principle for determining the capacity of a network for steady-state bifurcations is the determinant of Jacobian being zero. Thus, we only need to determine whether or not the constant term in the characteristic equation can be zero.
- The principle for determining the capacity of a network for oscillations via Hopf bifurcation is the Jacobian has a pair of pure imaginary eigenvalues. For a network with three nodes, the characteristic polynomial $P(\lambda)$ is order three. Let
$$P(\lambda) = \lambda^3 + a_1\lambda^2 + a_2\lambda + a_3. \tag{7}$$
Then the equation $P(\lambda) = 0$ has a pair of pure imaginary eigenvalues if and only if
$$a_2 > 0, \quad \text{and} \quad a_1a_2 - a_3 = 0.$$

Networks of family No. 2 in Figure 1. The corresponding vector field has the following form of
$$\begin{array}{rcl} \dot{x}_1 & = & -h^1(x_1) + f^1(x_2) \\ \dot{x}_2 & = & -h^2(x_2) + f^2(x_1). \end{array} \tag{8}$$

Multistability.

The equilibria for (8) satisfy:
$$h^1(x_1) = f^1(x_2) \quad \text{and} \quad h^2(x_2) = f^2(x_1).$$
Since h^i are monotonic, we have
$$h^2(x_2) = f^2((h^1)^{-1} \circ f^1(x_2)).$$

Note that h^i are monotonically increasing. According to the facts we stated in the beginning of the section, the network admits multiple equilibria if $f^2 \circ f^1$ is increasing; otherwise, the network admits at most one solution. That is, only positive feedback loops admit multistability.

Steady-state bifurcation.

The Jacobian of the system has the form of
$$J = \begin{pmatrix} -h_1^1 & f_2^1 \\ f_1^2 & -h_2^2, \end{pmatrix}$$
and $\det(J) = h_1^1 h_2^2 - f_2^1 f_3^1$. It follows from $\det(J)$ that positive feedback loops admit steady-state bifurcation.

Hopf bifurcation.

Note that $Trace(J) = -(h_1^1 + h_2^2) < 0$. Hence, it is impossible for the Jacobian having a pair of pure imaginary eigenvalues. Thus, no networks in this family admit Hopf bifurcation.

Family of networks No. 5 in Figure 1. The corresponding vector field has the following form

$$\begin{array}{rcl} \dot{x}_1 & = & -h^1(x_1) + f^1(x_2, x_3) \\ \dot{x}_2 & = & -h^2(x_2) + f^2(x_1) \\ \dot{x}_2 & = & -h^3(x_3) + f^3(x_1). \end{array} \tag{9}$$

Multistability.

The equilibria for (9) satisfy:

$$x_2 = (h^2)^{-1} \circ f^2(x_1), \quad x_3 = (h^3)^{-1} \circ f^3(x_1) \text{ and } h^1(x_1) = f^1(x_2, x_3).$$

Substituting the first two equalities into the third one, we obtain

$$h^1(x_1) = f^1(((h^2)^{-1} \circ f^2)(x_1), ((h^3)^{-1} \circ f^3)(x_1)).$$

Note that if $f^1 \circ f^2$ is increasing, we can arrange for the coupling between x_3 and x_1 to be weak enough such that the value of the right-hand side of (A) is determined by the first argument of the function f^1. That is, the monotonicity is determined by $f^1 \circ f^2$. Hence, the coupled feedback loops admit multistability if it contains at least one positive feedback loop.

Hopf bifurcation.

Using the coefficients of the characteristic polynomial of J, defined as in (7), we now check when do the conditions $a_2 > 0$ and $a_1 a_2 - a_3 = 0$ are satisfied. Observe that

$$a_2 = h_1^1 h_2^2 + h_1^1 h_3^3 + h_2^2 h_3^3 - f_2^1 f_1^2 - f_3^1 f_1^3.$$

$$\begin{array}{rcl} 0 = a_1 a_2 - a_3 & = & (h_1^1 + h_2^2)(h_1^1 h_2^2 + h_1^1 h_3^3 + h_2^2 h_3^3) + h_3^3(h_1^1 h_3^3 + h_2^2 h_3^3) \\ & & -(h_1^1 + h_3^3) f_3^1 f_1^3 - (h_1^1 + h_2^2) f_2^1 f_1^2 \end{array} \tag{10}$$

By $a_2 > 0$, we have

$$h_1^1 h_2^2 + h_1^1 h_3^3 + h_2^2 h_3^3 > f_2^1 f_1^2 + f_3^1 f_1^3. \tag{11}$$

Substituting the inequality (11) into equality (10) yields

$$(h_3^3 - h_2^2) f_3^1 f_1^3 > (h_3^3)^2 (h_2^2 + h_1^1).$$

On the other hand, the symmetric form of cells 2 and 3 implies that

$$(h_2^2 - h_3^3) f_2^1 f_1^2 > (h_2^2)^2 (h_3^3 + h_1^1).$$

Note that $h_i^i > 0$ since h_i are montonically increasing. In order to satisfy the above two inquality, $f_3^1 f_1^3$ and $f_2^1 f_1^2$ must have opposite sign.

Appendix B. **Steady-state bifurcations.** We illustrate how to classify codimension-one steady-state bifurcations from an equilibrium $X_0 = (x_1^0, x_2^0, x_3^0)$ for networks in Figure 1 using lyapunov-Schmidt reduction technique. We apply the technique to the vector fields of the networks in family No. 5 and we show that, generically, the systems undergo a saddle-node bifurcation. In Section B.1 we prove this result in a general framework. In Section B.2 we illustrate the calculations for a particular vector fields in the class of admissible vector fields for networks in family No. 5. This chosen vector field is used in [18] to draw numerically the bifurcation diagram.

B.1. **General result.** Consider the feedback loop in Figure 1, family No. 5. We write the system of admissible vector fields as

$$\dot{X} = F(X, \lambda), \tag{12}$$

where $\lambda \in \overline{\mathbf{R}^+}$ is a bifurcation parameter, $X = (x_1, x_2, x_3) \in \overline{\mathbf{R}^+}^3$, and $F(X, \lambda)$ is

$$\begin{aligned} & (F^1(X,\lambda), F^2(X,\lambda), F^3(X,\lambda)) \\ = \; & (-h^1(x_1) + f^1(x_2, x_3, \lambda), -h^2(x_2) + f^2(x_1, \lambda), -h^3(x_3) + f^3(x_1, \lambda)). \end{aligned} \tag{13}$$

We assume that the system (12)-(13) has an equilibrium $X_0 = (x_1^0, x_2^0, x_3^0)$. Also, we assume that the steady-state bifurcation occurs at (X_0, λ_0) with a zero eigenvalue with algebraic multiplicity one. Let J be the Jacobian matrix associate to (12)-(13) evaluated at (X_0, λ_0). Let

$$\begin{aligned} A &= -h_1^1(h^2h^3)^2 + f_{22}^1(h^3 f_1^2)^2 + f_{33}^1(h^2 f_1^3)^2 + f_{23}^1 f_1^2 f_1^3 h^2 (h^3)^2 \\ & \quad \text{and} \\ B &= h^2h^3 A + (h^3)^3 f_2^1[f_{11}^2 (h^2)^2 - h_2^2 (f_1^2)^2] + (h^2)^3 f_3^1[f_{11}^3 (h^3)^2 - \\ & \quad -(f_1^3)^2 h_3^3], \end{aligned} \tag{14}$$

where $h_j^i = \frac{\partial h^i}{dx_j}$ and $f_{jk}^i = \frac{\partial^2 f^i}{\partial x_j \partial x_k}$, with $i, j, k = 1, 2, 3$.

We claim that if

$$h^1h^2h^3 = h^2 f_3^1 f_1^3 + h^3 f_2^1 f_1^2, \qquad h^2h^3 f_\lambda^1 + h^3 f_2^1 f_\lambda^2 + h^2 f_3^1 f_\lambda^1 \neq 0, \qquad \text{and} \qquad B \neq 0 \tag{15}$$

holds then, generically a saddle-node bifurcation occurs for the system at (X_0, λ_0).

We next proof this claim. Note that a necessary and sufficient condition for steady-state bifurcation to occur is

$$\det J = 0.$$

A straightforward calculation shows that $\det J = 0$ if the first condition in the claim is satisfied. The simple eigenvalue of J implies that the reduced equation obtained by applying lyapunov-Schmidt reduction to (12)-(13) has the general form

$$g(x, \lambda) = 0, \tag{16}$$

where $x \in \mathbf{R}$, $g : \mathbf{R} \times \mathbf{R} \to \mathbf{R}$ is smooth, and $g(X_0, \lambda_0) = g_x(X_0, \lambda_0) = 0$. We next solve (16) near (X_0, λ_0). We claim that generically (16) satisfies

$$g_{xx}(X_0, \lambda_0) \neq 0 \neq g_\lambda(X_0, \lambda_0), \tag{17}$$

from which follows that saddle-node bifurcation occurs. To compute $g_{xx}(X_0, \lambda_0)$ and $g_x(X_0, \lambda_0)$ we use the following formulaes for the derivatives of the lyapunov-Schmidt reduction equation given, for example, in [13, Chapter 1, p. 33]

$$g_{xx}(X_0, \lambda_0) = \langle v_0^*, d^2F(v_0, v_0)\rangle \qquad \text{and} \qquad g_\lambda(X_0, \lambda_0) = \langle v_0^*, F_\lambda(X_0, \lambda_0)\rangle, \tag{18}$$

where $v_0 \in \ker J$, $v_0^* \in (\text{range } J)^\perp$. To compute these vectors we use J evaluated at (X_0, λ_0) and the first condition in (15). We found that

$$v_0 = (h^2h^3, h^3f_1^2, h^2f_1^3) \qquad \text{and} \qquad v_0^* = (h^2h^3, h^3f_2^1, h^2f_3^1).$$

A straightforward calculation using (13) and the formula for $g_\lambda(X_0, \lambda_0)$ in (18) shows that

$$g_\lambda(X_0, \lambda_0) = h^2h^3f_\lambda^1 + h^3f_2^1f_\lambda^2 + h^2f_3^1f_\lambda^1, \tag{19}$$

where $f_\lambda^i = \dfrac{\partial f^i}{\partial \lambda}$, $i = 1, 2, 3$. Note that generically the right-hand side of (19) is nonzero.

We next compute $g_{xx}(X_0, \lambda_0)$. Let $u = (u_1, u_2, u_3)$ and $v = (v_1, v_2, v_3)$ be vectors in $\mathbf{R}^3$. By definition

$$d^2F^l(u, v) = \sum_{i,j=1}^{3} \frac{\partial^2 F^l}{\partial x_i \partial x_j}(X_0, \lambda_0)u_iv_j. \tag{20}$$

Calculate $d^2F^l(v_0, v_0)$ using (20) and substitute into the first formula in (18) yields

$$g_{xx}(X_0, \lambda_0) = B,$$

where B is given in (14), which is generically nonzero.

B.2. **Particular example.** Here we illustrate the proof given in B.1 using a particular vector fields satisfying (12) and (13). That is, we assume the vector field is

$$\begin{cases} \dot{x_1} &= -x_1 + \lambda\dfrac{x_2^2 + x_3^2}{1 + x_2^2 + x_3^2} \equiv F^1 \\ \dot{x_2} &= -x_2 + \lambda\dfrac{x_1^2}{1 + x_1^2} \equiv F^2 \\ \dot{x_3} &= -x_3 + \lambda\dfrac{x_1^2}{1 + x_1^2} \equiv F^3, \end{cases} \tag{21}$$

where the x_i's are one-dimensional and λ is the bifurcations parameter and represents the coupling strength. We observe that, as mentioned before, in [18] the authors use this vector field to draw numerically the bifurcation diagram.

Note that the equilibria of (21) satisfy

$$x_2 = x_3 = \frac{\lambda x_1^2}{1 + x_1^2} \qquad \text{and} \qquad x_1 = \frac{2\lambda x_2^2}{1 + 2x_2^2}.$$

In particular $(x_1, x_2, x_3, \lambda) = (0, 0, 0, \lambda)$ is an equilibrium for the system, which we call *trivial*. The Jacobian evaluated at any equilibrium point has the form

$$J = \begin{pmatrix} -1 & a & a \\ b & -1 & 0 \\ b & 0 & -1 \end{pmatrix}, \tag{22}$$

with

$$a = \frac{2\lambda x_2}{(1 + 2x_2^2)^2} \qquad \text{and} \qquad b = \frac{2\lambda x_1}{(1 + x_1^2)^2}. \tag{23}$$

When considering the trivial equilibrium it follows that $J = -I$, where I denote the 3×3 identity matrix. Thus, $\det J = -1 \neq 0$. Hence, the implicit function theorem guarantees the existence of an unique branch of equilibria parameterized by λ, which, after a $\lambda-$dependent affine linear change in coordinates, we can assume is $(0, 0, 0, \lambda)$. The equilibria on this branch are stable.

We next classify steady-state bifurcations from a fixed nontrivial equilibrium $X_0 = (x_1^0, x_2^0, x_3^0)$ for (21). This type of bifurcation occurs in the system if and only if

$$\det J = -1 + 2ab = 0 \qquad \Leftrightarrow \qquad 2ab = 1 \tag{24}$$

holds. We assume that the bifurcation occurs at (X_0, λ_0) with $\lambda_0 \neq 0$ since, otherwise $\det J = -1 \neq 0$ and we would have the unique branch of trivial solutions identified previously. A straightforward calculation shows that the eigenvalues of J are -1, $1 + \sqrt{2ab}$, and $1 - \sqrt{2ab}$, which are real and distinct. Hence, when (24) holds J has a simple zero eigenvalue. Under this assumptions we claim that generically the bifurcation is of type saddle-node. This follows directly from the fact that the system (21) has $\mathbf{Z}_2 = \langle (2, 3) \rangle$ symmetry that acts trivially on kernel of J at (X_0, λ_0). The simple eigenvalue of J implies that the reduced equation obtained by applying lyapunov-Schmidt reduction to (21) has the general form given in (16).

We next solve (16) near (X_0, λ_0) and show that saddle-node bifurcation occurs in the system, that is, condition (17) holds. It follows from (22) and (24) that $v_0 = (1, b, b)$, for which the action of $\mathbf{Z}_2$ symmetry acts trivially, and $v_0^* = (1, a, a)$. A straightforward calculation using (12), with coordinate functions defined as in (21), and the formula for $g_\lambda(X_0, \lambda_0)$ in (18) shows that

$$g_\lambda(X_0, \lambda_0) = \frac{2x_2^2}{1 + 2x_2^2} + \frac{2ax_1^2}{1 + x_1^2} > 0,$$

with a given in (23). Note that the inequality following from the fact that $a > 0$. We next compute $g_{xx}(X_0, \lambda_0)$. Since $v_0^* = (1, a, a)$, it follows that

$$g_{xx}(X_0, \lambda_0) = d^2F^1(v_0, v_0) + ad^2F^2(v_0, v_0) + ad^2F^3(v_0, v_0). \tag{25}$$

Calculate $d^2F^l(v_0, v_0)$ using (20) and substitute into (25) yields

$$g_{xx} = 4a\lambda_0(C + D),$$

with $a > 0$ given in (23) and

$$C = \frac{a(1 - 4x_2^2)}{(1 + 2x_2^2)^3}, \qquad D = \frac{1 - 3x_1^2}{(1 + x_1^2)^3}.$$

Observe that generically $C + D \neq 0$ and since a, $\lambda_0 > 0$ it follows that generically $g_{xx} \neq 0$ as claimed.

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