MULTISTABILITY OF SMALL REACTION NETWORKS

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Abstract. For three typical sets of small reaction networks (networks with two reactions, one irreversible and one reversible reaction, or two reversible-reaction pairs), we completely answer the challenging question: what is the smallest subset of all multistable networks such that any multistable network outside of the subset contains either more species or more reactants than any network in this subset?

Key words. chemical reaction networks, mass-action kinetics, multistationarity, multistability

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1. Introduction. For the dynamical systems that arise from biochemical reaction networks, we ask the following question:

QUESTION 1.1. Given a class of networks with the same number of irreversible reactions and the same number of reversible-reaction pairs, what is the smallest nonempty subset of multistable networks such that any multistable network outside of the subset has either more species or more reactants than any network from the subset? Here, we define the number of reactants as the maximum sum of stoichiometric coefficients in the reactant complexes (see Definition 2.3).

We will formally state this question in Section 2.3, see Question 2.4. The above question is motivated by the multistationarity (multistability) problem of biochemical reaction systems, which is crucial for understanding basic phenomena such as decision-making process in cellular signaling [3, 9, 23, 5]. Given a network, we pursue rate constants such that the corresponding dynamical system arising under mass-action kinetics has at least two (stable) positive steady states in the same stoichiometric compatibility class. Mathematically, one needs to identify a value or an open region in the parameter space for which a parametric semi-algebraic system has at least two real solutions, which is a fundamental problem in computational algebraic geometry [14, 6]. It is well-known that networks with only reaction admit no multistationarity/multistability. So, for Question 1.1, the first non-trivial case to study is the class of networks with two reactions (possibly reversible). It is implied by [13] that for the networks with two pairs of reversible reactions, the smallest nonempty subclass of multistable networks is the set with a single network “0 ⇔ X₁, 2X₁ ⇔ 3X₁”.

That means for any other network with two reversible-reaction pairs, if it admits multistability, either it has at least 2 species, or the number of reactants is at least 4. In this paper, our main contributions are complete answers to Question 1.1 for the networks with exactly two reactions (see Theorem 2.5) and those with one irreversible reaction and one reversible reaction (see Theorem 2.6).

Our main focus is the multistability problem. Generally, multistability is a much more difficult problem than multistationarity because the standard algebraic tool for studying stability (Routh-Hurwitz criterion [11], or alternatively Liénard-Chipart criterion [7]) is computationally challenging (e.g., [17], [21]). Fortunately, for the networks with one-dimensional stoichiometric subspaces, we can determine stability by checking the trace of the Jacobian matrix (see Lemma 3.2). Using the simpler criteria,
we employ elimination method (from algebraic geometry), and then we prove an upper bound (and a lower bound for the networks such that the nondegeneracy conjecture [13, Conjecture 2.3] is true) for the maximum number of stable positive steady states in terms of the maximum number of positive steady states (see Theorems 3.14 and 3.15), which shows a multistable network admits at least three positive steady states, and so the number of reactants should be at least three (in fact, for two-reaction networks, the number of reactants should be at least four, see Theorem 4.9). A recent study on at-most-bimolecular networks [16] supports our result. These results extend [13, Theorem 3.6 2(c)], which is for one-species networks, to two-reaction networks and to two-species networks with one irreversible and one reversible reaction, or with two pairs of reversible reactions. Remark that these results are based on a sign condition (see Theorem 3.5), which also provides one way to determine multistationarity (by checking if the determinant of the Jacobian matrix changes sign) for small networks with one-dimensional stoichiometric subspaces (see Corollary 3.13). There have been a long list of such criterion (without or with a steady-state parametrization), see [4, 19, 22, 1, 14, 6, 8]. One criteria in the list based on degree theory [8, Theorem 3.12] requires the networks to admit no boundary steady states, which can not be directly applied to two-reaction networks since if a two-reaction network admits multistationarity, then it must admit boundary steady states (see Theorem 4.8).

This work can be viewed as one step toward an ambitious goal: a complete classification of multistable networks with one-dimensional stoichiometric subspaces. As the first step toward the big goal, Joshi and Shiu [13] solved the multistationarity problem for small networks with only one species or up to two reactions (possibly reversible). Later, Shiu and de Wolff [20] extended these results to nondegenerate multistationarity for two-species networks with two reactions (possibly reversible). The idea of studying small networks is inspired by the fact that nondegenerate multistationarity can be lifted from small networks to related large networks [12, 2]. Here, our contribution is straightforward: one can directly read multistable networks with few species and few reactants from the two main results Theorem 2.5 and Theorem 2.6. For two-reaction networks with up to four reactants and up to three species, there are in fact only two kinds of networks (but infinitely many) that are multistable. For instance, by Theorem 2.5, we directly see the network “\(X_1 \rightarrow X_2 + X_3, 2X_1 + X_2 + X_3 \rightarrow 3X_1\)” admits no multistability. And, for the networks with one irreversible and one reversible reaction, if there are up to three reactants and up to two species, then only four kinds of networks are multistable.

The rest of this paper is organized as follows. In Section 2, we introduce mass-action kinetics systems arising from reaction networks. We formally state our problem and present the main results in Section 2.3. In Section 3, for the small networks with one-dimensional stoichiometric subspaces, we provide a sign condition (see Theorem 3.5), which reveals the relationship between the maximum number of positive steady states and the maximum number of stable positive steady states (see Theorems 3.14 and 3.15). In Section 4, we study networks with exactly two reactions. We prove a list of necessary conditions for a two-reaction network to admit multistability (for instance, see Theorems 4.8 and 4.9). Based on these results, for the set of all two-reaction networks, we find the smallest subset of all multistable networks such that any multistable network contains either more species or more reactants than any network in this subset (see the proof of Theorem 2.5). We extend these results for networks with reversible reactions in Section 5 (see the proof of Theorem 2.6). Finally, we end up this paper with open problems inspired by Theorem 3.15, see Section 6.
2. Background.

2.1. Chemical reaction networks. In this section, we briefly recall the standard notions and definitions on reaction networks, see [6, 13] for more details. A reaction network \( G \) (or network for short) consists of a set of \( s \) species \( \{X_1, X_2, \ldots, X_s\} \) and a set of \( m \) reactions:

\[
\alpha_{ij}X_1 + \cdots + \alpha_{sj}X_s \rightarrow \beta_{ij}X_1 + \cdots + \beta_{sj}X_s, \text{ for } j = 1, 2, \ldots, m,
\]

where all \( \alpha_{ij} \) and \( \beta_{ij} \) are non-negative integers, and \( (\alpha_{1j}, \ldots, \alpha_{sj}) \neq (\beta_{1j}, \ldots, \beta_{sj}) \). We call the \( s \times m \) matrix with \((i, j)\)-entry equal to \( \beta_{ij} - \alpha_{ij} \) the stoichiometric matrix of \( G \), denoted by \( N \). We call the image of \( N \) the stoichiometric subspace, denoted by \( S \).

We denote by \( x_1, x_2, \ldots, x_s \) the concentrations of the species \( X_1, X_2, \ldots, X_s \), respectively. Under the assumption of mass-action kinetics, we describe how these concentrations change in time by following system of ODEs:

\[
\dot{x} = f(x) := N \cdot \begin{pmatrix}
\kappa_1 x_1^{\alpha_{11}} x_2^{\alpha_{12}} \cdots x_s^{\alpha_{1s}} \\
\kappa_2 x_1^{\alpha_{21}} x_2^{\alpha_{22}} \cdots x_s^{\alpha_{2s}} \\
\vdots \\
\kappa_m x_1^{\alpha_{m1}} x_2^{\alpha_{m2}} \cdots x_s^{\alpha_{ms}}
\end{pmatrix},
\]

where \( x \) denotes the vector \((x_1, x_2, \ldots, x_s)\), and each \( \kappa_j \in \mathbb{R}_{>0} \) is called a rate constant. By considering the rate constants as an unknown vector \( \kappa = (\kappa_1, \kappa_2, \ldots, \kappa_m) \), we have polynomials \( f_i \in \mathbb{Q}[\kappa, x] \), for \( i = 1, 2, \ldots, s \).

A conservation-law matrix of \( G \), denoted by \( W \), is any row-reduced \( d \times s \)-matrix whose rows form a basis of \( S^\perp \), where \( d := s - \text{rank}(N) \) (note here, \( \text{rank}(W) = d \)). Our system (2.2) satisfies \( W \dot{x} = 0 \), and both the positive orthant \( \mathbb{R}_{>0}^s \) and its closure \( \mathbb{R}_{\geq0}^s \) are forward-invariant for the dynamics. Thus, a trajectory \( x(t) \) beginning at a nonnegative vector \( x(0) = x^0 \in \mathbb{R}_{\geq0}^s \) remains, for all positive time, in the following stoichiometric compatibility class with respect to the total-constant vector \( c := Wx^0 \in \mathbb{R}^d \):

\[
P_c := \{ x \in \mathbb{R}_{\geq0}^s \mid Wx = c \}.
\]

That means \( P_c \) is forward-invariant with respect to the dynamics (2.2).

In this work, we mainly focus on the three families of small networks defined as

\[
G_0 := \{ \text{the networks with exactly two reactions, i.e., } m = 2 \text{ in (2.1)} \},
\]

\[
G_1 := \{ \text{the networks with one irreversible and one reversible reaction} \},
\]

\[
G_2 := \{ \text{the networks with two of reversible-reaction pairs} \}.
\]

We denote the union \( \bigcup_{i=0}^2 G_i \) simply by \( G \). Also, we simplify/clarify our notation (2.1) for reversible reactions. For any \( G \in G_1 \), we denote it by

\[
\Sigma^s x_i \iff \Sigma^s_{i=1} \alpha_{i1} X_i, \quad \Sigma^s x_i \implies \Sigma^s_{i=1} \beta_{i2} X_i,
\]

and for any \( G \in G_2 \), we denote it by

\[
\Sigma^s x_i \iff \Sigma^s_{i=1} \alpha_{i1} X_i, \quad \Sigma^s x_i \implies \Sigma^s_{i=1} \beta_{i2} X_i.
\]
2.2. Steady states. A steady state of (2.2) is a concentration-vector \( x^* \in \mathbb{R}_{\geq 0}^n \) at which \( f(x) \) on the right-hand side of the ODEs (2.2) vanishes, i.e., \( f(x^*) = 0 \). If a steady state \( x^* \) has all strictly positive coordinates (i.e., \( x^* \in \mathbb{R}_{> 0}^n \)), then we call \( x^* \) a positive steady state. If a steady state \( x^* \) has zero coordinates (i.e., \( x^* \in \mathbb{R}_{\geq 0}^n \)), then we call \( x^* \) a boundary steady state. We say a steady state \( x^* \) is nondegenerate if \( \text{im}(\text{Jac}_f(x^*)) = S \), where \( \text{Jac}_f(x^*) \) denotes the Jacobian matrix of \( f \), with respect to \( (w.r.t.) x \), at \( x^* \). A nondegenerate steady state \( x^* \) is Liapunov stable if for any \( \epsilon > 0 \) and for any \( t_0 > 0 \), there exists \( \delta > 0 \) such that \( \| x(t_0) - x^* \| < \delta \) implies \( \| x(t) - x^* \| < \epsilon \) for any \( t \geq t_0 \). A Liapunov stable steady state \( x^* \) is locally asymptotically stable if there exists \( \delta > 0 \) such that \( \| x(t_0) - x^* \| < \delta \) implies \( \lim_{t \to \infty} x(t) = x^* \). A nondegenerate steady state \( x^* \) is exponentially stable (or, simply stable in this paper) if all non-zero eigenvalues of \( \text{Jac}_f(x^*) \) have negative real parts. Note that if a steady state is exponentially stable, then it is locally asymptotically stable [18].

Suppose \( N \in \mathbb{Z}_{\geq 0} \). A network admits \( N \) (nondegenerate) positive steady states if for some rate-constant vector \( \kappa \) and for some total-constant vector \( c \), it has \( N \) (nondegenerate) positive steady states at the same stoichiometric compatibility class \( P_c \). A network admits \( N \) stable positive steady states if for some rate-constant vector \( \kappa \) and for some total-constant vector \( c \), it has \( N \) stable positive steady states at the same stoichiometric compatibility class \( P_c \).

The maximum number of positive steady states of a network \( G \) is

\[
\text{cap}_{\text{pos}}(G) := \max\{N \in \mathbb{Z}_{\geq 0} \cup \{+\infty\} | G \text{ admits } N \text{ positive steady states}\}.
\]

The maximum number of nondegenerate positive steady states of a network \( G \) is

\[
\text{cap}_{\text{nondeg}}(G) := \max\{N \in \mathbb{Z}_{\geq 0} \cup \{+\infty\} | G \text{ admits } N \text{ nondegenerate positive steady states}\}.
\]

The maximum number of stable positive steady states of a network \( G \) is

\[
\text{cap}_{\text{stab}}(G) := \max\{N \in \mathbb{Z}_{\geq 0} \cup \{+\infty\} | G \text{ admits } N \text{ stable positive steady states}\}.
\]

It is obvious that if \( \hat{G} \) has the form of \( G \), then \( \text{cap}_{\text{pos}}(\hat{G}) = \text{cap}_{\text{pos}}(G) \), \( \text{cap}_{\text{nondeg}}(\hat{G}) = \text{cap}_{\text{nondeg}}(G) \), and \( \text{cap}_{\text{stab}}(\hat{G}) = \text{cap}_{\text{stab}}(G) \).

We say a network admits multistationarity if \( \text{cap}_{\text{pos}}(G) \geq 2 \). We say a network admits nondegenerate multistationarity if \( \text{cap}_{\text{nondeg}}(G) \geq 2 \). We say a network admits multistability if \( \text{cap}_{\text{stab}}(G) \geq 2 \).

2.3. Problem statement and Main Results.

Definition 2.3. For a non-negative integer \( K \), a network \( G \) with reactions defined in (2.1) is at-most-\( K \)-reactant if for all \( j \in \{1, \ldots, m\} \), we have \( \sum_{k=1}^{s} \alpha_{kj} \leq K \), and we say \( G \) is \( K \)-reactant (or, the number of reactants of \( G \) is \( K \)) if \( G \) is at-most-\( K \)-reactant and there exists \( j \in \{1, \ldots, m\} \) such that \( \sum_{k=1}^{s} \alpha_{kj} = K \).

For \( i \in \{0, 1, 2\} \), define \( M_i \) as the set of subsets of \( \mathcal{G}_i \) such that every \( \mathcal{H} \in M_i \) satisfies the two conditions:
(i) for any $G \in \mathcal{H}$, $G$ admits multistability, and
(ii) for any $G \in \mathcal{G}_1 \setminus \mathcal{H}$ and for any $\hat{G} \in \mathcal{H}$, if $G$ admits multistability, then $G$
contains either more species or more reactants than $\hat{G}$.

**Question 2.4** (Formal version of Question 1.1). For $i \in \{0, 1, 2\}$, find $\mathcal{H}^* \in \mathcal{M}_i$
such that $\mathcal{H}^* \neq \emptyset$ and for any $H \in \mathcal{M}_i$, we have $\mathcal{H}^* \subseteq \mathcal{H}$.

We provide a complete answer to Question 2.4, see Theorem 2.5, Theorem 2.6 and Theorem 2.7.

**Theorem 2.5.** Given $G \in \mathcal{G}_0$, if $G$ has up to 3 species and $G$ is at-most-4-reactant, then $G$
admits multistability if and only if $G$ has the form of one of the two networks $(2.8)$ and $(2.9)$ below

$$(2.8) \quad X_1 + 3X_2 \rightarrow 4X_2 + X_3, \quad X_2 + X_3 \rightarrow X_1;$$

$$(2.9) \quad X_1 + 2X_2 + X_3 \rightarrow \beta_{21}X_2, \quad 3X_3 \rightarrow \beta_{12}X_1 + \beta_{22}X_2 + \beta_{32}X_3,$$

where $\beta_{21} \in \{0, 1\}$, $\beta_{12} \in \mathbb{Z}_{>0}$, $\beta_{22} = \beta_{12}(2 - \beta_{21})$ and $\beta_{32} = \beta_{12} + 2$.

Theorem 2.5 means for $\mathcal{G}_0$, the answer to Question 2.4 is

$$\mathcal{H}^* := \{G \text{ has the form of the network (2.8), or the network (2.9)}\}.$$

In fact, Theorem 2.5 implies that the above set satisfies the conditions (i) and (ii).

For any $\mathcal{H} \in \mathcal{M}_i$, if there exists $G \in \mathcal{H}^*$ such that $G \notin \mathcal{H}$, then by the condition (ii),
there exists a multistable network $\hat{G} \in \mathcal{H}$ such that $G$ has either less than 3 species or
less than 4 reactants, which is a contradiction to Theorem 2.5. So, we definitely have $\mathcal{H}^* \subseteq \mathcal{H}$.
Similarly, one can understand why Theorems 2.6 and 2.7 below answer Question 2.4 for $\mathcal{G}_1$ and $\mathcal{G}_2$, respectively.

**Theorem 2.6.** Given $G \in \mathcal{G}_1$, if $G$ has up to 2 species and $G$ is at-most-3-reactant, then $G$
admits multistability if and only if $G$ has the form of one of the networks listed in Rows (7)–(10) of Table 4.

**Theorem 2.7.** For $G \in \mathcal{G}_2$, if $G$ has only one species and $G$ is at-most-3-reactant, then $G$
admits multistability if and only if $G$ has the form of the network

$$(2.10) \quad 0 \iff X_1, \quad 2X_1 \iff 3X_1.$$

It is straightforward to prove Theorem 2.7 by Theorem 3.14 (see Section 3.3) and
[13, Theorem 3.6]. We provide the details in “SM.pdf” (Table 5). Here, our main
contributions are Theorem 2.5 and Theorem 2.6. See the proofs in Section 4.2 and
Section 5. Note that for each set $\mathcal{G}_i$, an ambitious goal is to find the subset of all
multistable network, which can be viewed as the “largest” element in $\mathcal{M}_i$. Our work
provides one way to achieve the goal by detecting multistable networks when the
number of species and the number of reactants are restricted.

**3. Small networks with one-dimensional stoichiometric subspaces.**

**3.1. Stability.**

**Assumption 3.1.** For any $G \in \mathcal{G}$ with reactions defined in (2.1), by the definition of reaction network, we know $(\alpha_{11}, \ldots, \alpha_{s1}) \neq (\beta_{11}, \ldots, \beta_{s1})$. Without loss of
generality, we would assume $\beta_{11} - \alpha_{11} \neq 0$ throughout this paper.

**Lemma 3.2.** For any $G \in \mathcal{G}$, if the stoichiometric subspace of $G$ is one-dimensional,
then for a nondegenerate steady state $x^*$, it is stable if and only if $\sum_{i=1}^{s} \frac{\partial L}{\partial x_i} |_{x=x^*} < 0.$
Proof. Since the stoichiometric subspace of $G$ is one-dimensional, there exists $\lambda \in \mathbb{R}$ such that

$$
\begin{pmatrix}
\beta_{12} - \alpha_{12} \\
\vdots \\
\beta_{s2} - \alpha_{s2} \\
\end{pmatrix} = -\lambda \begin{pmatrix}
\beta_{11} - \alpha_{11} \\
\vdots \\
\beta_{s1} - \alpha_{s1} \\
\end{pmatrix}.
$$

We substitute (3.1) into $f(x)$ in (2.2), and we have

$$
(\beta_{11} - \alpha_{11}) f_i = (\beta_{11} - \alpha_{11}) f_i, \text{ for } i = 2, \ldots, s.
$$

(For instance, if $G \in \mathcal{G}_1$, then

$$
(3.3) \quad f_i = \kappa_1 (\beta_{11} - \alpha_{11}) \Pi_{k=1}^{s} \alpha_{k1} - \lambda \kappa_2 (\beta_{11} - \alpha_{11}) \Pi_{k=1}^{s} \alpha_{k2}, \quad i = 1, \ldots, s.
$$

So, the equality (3.2) directly follows from (3.3). If $G \in \mathcal{G}_1$ or $\mathcal{G}_2$, we can similarly derive (3.2). By (3.2), the matrix $\text{Jac}_f(x^*)$ has rank 1, and so, it has at most one non-zero eigenvalue. Note also $\text{Jac}_f(x^*)$ has at least one non-zero eigenvalue since $x^*$ is nondegenerate. Therefore, there is only one non-zero eigenvalue, which is equal to the trace of the Jacobian matrix $\text{Jac}_f(x^*)$.

Lemma 3.3. [13, Lemma 4.1, Theorem 5.8, Theorem 5.12] For $G \in \mathcal{G}$, if $G$ admits multistationarity, then there exists $\lambda \in \mathbb{R}\setminus\{0\}$ such that the equality (3.1) holds, and, additionally, if $G \in \mathcal{G}_0$, then the scalar $\lambda$ is positive.

Corollary 3.4. For $G \in \mathcal{G}$, if $G$ admits multistationarity, then the stoichiometric subspace of $G$ is one-dimensional.

3.2. Sign condition. For any $G \in \mathcal{G}$, suppose $f(x) = (f_1(x), \ldots, f_s(x))$ is defined as (2.2), and suppose the stoichiometric subspace of $G$ is one-dimensional. Define the system augmented with the conservation laws:

$$
(3.4) \quad h_1 := f_1, \quad h_i := (\beta_{11} - \alpha_{11}) x_1 - (\beta_{11} - \alpha_{11}) x_i - c_{i-1}, \quad 2 \leq i \leq s.
$$

Theorem 3.5. Given $G \in \mathcal{G}$, suppose the stoichiometric subspace of $G$ is one-dimensional. If for a rate-constant vector $x^*$ and a total-constant vector $c^*$, $G$ has exactly $N$ distinct positive steady states $x^{(1)}, \ldots, x^{(N)}$, where $x^{(1)}, \ldots, x^{(N)}$ are ordered according to their first coordinates (i.e., $x^{(1)}_1 < \ldots < x^{(N)}_1$), and all positive steady states are nondegenerate, then $|\text{Jac}_h(x^{(i)})| |\text{Jac}_h(x^{(i+1)})|<0$ for $i \in \{1, \ldots, N-1\}$.

The goal of this subsection is to prove Theorem 3.5. We first prepare some lemmas. In fact, Theorem 3.5 directly follows from Lemma 3.6, Lemma 3.8, Lemma 3.10 and Lemma 3.11.

Lemma 3.6. Let $g(z) := a_n z^n + \cdots + a_1 z + a_0$ be a univariate polynomial in $\mathbb{R}[z]$. If the equation $g(z) = 0$ has exactly $r$ ($r \geq 2$) distinct real roots, say $z_1 < \cdots < z_r$, and if $g'(z_i) \neq 0$ for $i \in \{1, \ldots, r\}$, then we have $g'(z_i) g'(z_{i+1}) < 0$ for $i \in \{1, \ldots, r-1\}$.

Proof. Fix any $i \in \{1, \ldots, r-1\}$, let $h(z)$ be the univariate polynomial such that $g(z) = (z - z_i)(z - z_{i+1}) h(z)$. Note that

$$
g'(z) = (z - z_{i+1}) h(z) + (z - z_i) h(z) + (z - z_i)(z - z_{i+1}) h'(z).
$$

So, $g'(z_i) g'(z_{i+1}) = - (z_{i+1} - z_i)^2 h(z_i) h(z_{i+1})$. If $g'(z_i) g'(z_{i+1}) > 0$, then we have $h(z_i) h(z_{i+1}) < 0$. Notice that $h(z)$ is a continuous function, so there exists $z_0 \in (z_i, z_{i+1})$ such that $h(z_0) = 0$. We know $z_0 \neq z_i$ for $i \in \{1, \ldots, r\}$, which is a contradiction to the hypothesis that $g(z) = 0$ has exactly $r$ distinct roots. Therefore, we definitely have $g'(z_i) g'(z_{i+1}) < 0$. □
Lemma 3.7. The determinant of Jacobian matrix of \( h \) defined in (3.4) w.r.t \( x \) (denoted by \( |\text{Jac}_h| \)) is equal to

\[
(\alpha_{11} - \beta_{11})^{s-2} \sum_{i=1}^{s} (\alpha_{i+1} - \beta_{i+1}) \frac{\partial f_1}{\partial x_i},
\]

which is also equal to

\[
(\alpha_{11} - \beta_{11})^{s-1} \sum_{i=1}^{s} \frac{\partial f_i}{\partial x_i}.
\]

Proof. Let \( \text{Jac}_h \) denote the Jacobian matrix of \( h \) w.r.t \( x \):

\[
\text{Jac}_h = \begin{bmatrix}
\frac{\partial f_1}{\partial x_1} & \frac{\partial f_1}{\partial x_2} & \ldots & \frac{\partial f_1}{\partial x_s} \\
\beta_{21} - \alpha_{21} & \alpha_{11} - \beta_{11} & 0 & \ldots & 0 \\
\beta_{31} - \alpha_{31} & 0 & \alpha_{11} - \beta_{11} & \ldots & 0 \\
\vdots & \vdots & \vdots & \ddots & \vdots \\
\beta_{s1} - \alpha_{s1} & 0 & 0 & \ldots & \alpha_{11} - \beta_{11}
\end{bmatrix}.
\]

Below, we prove (3.5) by induction. First, for \( s = 2 \), we have

\[
|\text{Jac}_h| = \begin{vmatrix}
\beta_{21} - \alpha_{21} & \alpha_{11} - \beta_{11} \\
\beta_{31} - \alpha_{31} & 0 & \alpha_{11} - \beta_{11} & \ldots & 0 \\
\vdots & \vdots & \vdots & \ddots & \vdots \\
\beta_{s1} - \alpha_{s1} & 0 & 0 & \ldots & \alpha_{11} - \beta_{11}
\end{vmatrix}.
\]

Second, we assume for \( s = n \) \((n \geq 2)\), the conclusion holds. For \( s = n + 1 \), we apply Laplace expansion to \( |\text{Jac}_h| \) w.r.t the last row, and we get

\[
|\text{Jac}_h| = (\alpha_{11} - \beta_{11})^{n-1} (\alpha_{n+1,1} - \beta_{n+1,1}) \frac{\partial f_1}{\partial x_{n+1}} + (\alpha_{11} - \beta_{11})^{n-1} \sum_{i=1}^{n} (\alpha_{i+1,1} - \beta_{i+1,1}) \frac{\partial f_i}{\partial x_i}
\]

\[
= (\alpha_{11} - \beta_{11})^{n-1} \sum_{i=1}^{n+1} (\alpha_{i+1,1} - \beta_{i+1,1}) \frac{\partial f_i}{\partial x_i}.
\]

Finally, by Lemma 3.7 and the equalities (3.2), we have (3.6).

For the system \( h \) defined in (3.4), define

\[
g(x_1) := h_1(x_1, \ldots, x_s)|_{x_2 = \frac{\beta_{21} - \alpha_{21}}{\pi_{11} - \pi_{12}} x_1, x_3 = \frac{\beta_{31} - \alpha_{31}}{\pi_{11} - \alpha_{11}} x_1, \ldots, x_s = \frac{\beta_{s1} - \alpha_{s1}}{\pi_{11} - \alpha_{11}} x_1}.
\]

Lemma 3.8. For the system \( h(x) \) (3.4) and the polynomial \( g(x_1) \) (3.7), if \( x^* \) is a solution to \( h_1(x^*) = \ldots = h_s(x^*) = 0 \), then

\[
(\alpha_{11} - \beta_{11})^{s-1} g'(x_1^*) = |\text{Jac}_h(x^*)|.
\]

Proof. By (3.7), and by the long division, we have \( g(x_1) = h_1 + \frac{1}{\pi_{11} - \alpha_{11}} \sum_{i=2}^{s} q_i h_i \),
where \( q_1, \ldots, q_s \) are polynomials in \( \mathbb{R}[x_1, \ldots, x_s] \). So,

\[
g'(x_1^*) = \frac{\partial h_1}{\partial x_1}(x^*) + \frac{1}{\beta_{11} - \alpha_{11}} \sum_{i=2}^{s} q_i(x^*) \frac{\partial h_i}{\partial x_1}(x^*)
\]

(3.9)

\[
0 = \frac{\partial h_1}{\partial x_i}(x^*) + \frac{1}{\beta_{11} - \alpha_{11}} q_i(x^*) \frac{\partial h_i}{\partial x_i}(x^*),
\]

(3.10)

By (3.10), we have \( q_i(x^*) = \frac{\partial h_i}{\partial x_i}(x^*) \). So, by (3.9), we have

\[
g'(x_1^*) = \frac{\partial h_1}{\partial x_1}(x^*) + \frac{1}{\beta_{11} - \alpha_{11}} \sum_{i=2}^{s} \frac{\partial h_1}{\partial x_i}(x^*) (\beta_{11} - \alpha_{11})
\]

(3.11)

Note that \( h_1 = f_1 \) (see (3.4)). By Lemma 3.7 and (3.11), we have (3.8).

**Lemma 3.9.** Given \( G \in \mathcal{G} \), suppose the stoichiometric subspace of \( G \) is one-dimensional. If for a rate-constant vector \( \kappa^* \) and a total-constant vector \( c^* \), \( G \) has a positive steady state \( x^* \), then the first coordinate \( x_1^* \) is contained in the open interval

\[
I := \cap_{i=2}^{s} I_i \quad \text{where} \quad I_i := \begin{cases} 
\left( -\frac{\alpha_{1i}}{\beta_{1i} - \alpha_{1i}}, +\infty \right) & \text{if} \ \frac{\beta_{11} - \alpha_{11}}{\beta_{11} - \alpha_{1i}} > 0 \\
(0, +\infty) & \text{if} \ \frac{\beta_{11} - \alpha_{11}}{\beta_{11} - \alpha_{1i}} = 0 \\
(0, -\frac{\alpha_{1i}}{\beta_{11} - \alpha_{11}}) & \text{if} \ \frac{\beta_{11} - \alpha_{11}}{\beta_{11} - \alpha_{1i}} < 0
\end{cases}
\]

(3.12)

**Proof.** By (3.4), for any \( i \ (2 \leq i \leq s) \), \( x_i^* = \frac{\beta_{1i} - \alpha_{1i}}{\beta_{11} - \alpha_{1i}} x_1^* - \frac{\alpha_{1i}}{\beta_{11} - \alpha_{1i}} > 0 \). So \( x_1^* \) is contained in the interval \( I \) defined in (3.12).

**Lemma 3.10.** Given \( G \in \mathcal{G} \), suppose the stoichiometric subspace of \( G \) is one-dimensional. If for a rate-constant vector \( \kappa^* \) and a total-constant vector \( c^* \), \( G \) has exactly \( N \) distinct positive steady states \( x_1^{(1)}, \ldots, x_1^{(N)} \), where \( x_1^{(1)}, \ldots, x_1^{(N)} \) are ordered according to their first coordinates \( (i.e., x_1^{(1)} < \ldots < x_1^{(N)}) \), then all \( x_1^{(1)}, \ldots, x_1^{(N)} \) are roots to \( g(x_1) = 0 \), and for any \( 1 \leq i \leq N - 1 \), there is no other real root to \( g(x_1) = 0 \) between \( x_1^{(i)} \) and \( x_1^{(i+1)} \).

**Proof.** By (3.4) and (3.7), all \( x_1^{(1)}, \ldots, x_1^{(N)} \) are roots to \( g(x_1) = 0 \). By Lemma 3.9, we have \( x_1^{(1)}, \ldots, x_1^{(N)} \in I \) (see (3.12)). Hence, if \( g(x_1) = 0 \) has a real solution \( x_1^* \) between the two solutions \( x_1^{(i)} \) and \( x_1^{(i+1)} \), then \( x_1^* \in I \). For \( j = 2, \ldots, s \), let \( x_j^* = \frac{\beta_{1j} - \alpha_{1j}}{\beta_{11} - \alpha_{11}} x_1^* - \frac{\alpha_{1j}}{\beta_{11} - \alpha_{11}} \). Then \( x^* \) is also a positive steady state, and it is different from \( x_1^{(1)}, \ldots, x_1^{(N)} \), which is a contradiction to the hypothesis that \( G \) has exactly \( N \) distinct positive steady states.

**Lemma 3.11.** Given \( G \in \mathcal{G} \), suppose the stoichiometric subspace of \( G \) is one-dimensional. If a steady state \( x^* \) is nondegenerate, then \( |\text{Jac}_h(x^*)| \neq 0 \).

**Proof.** Let \( N_1 := (\beta_{11} - \alpha_{11}, \ldots, \beta_{1s} - \alpha_{1s})^T \) (recall we assume \( \beta_{11} - \alpha_{11} \neq 0 \)). If \( |\text{Jac}_h(x^*)| = 0 \), then by (3.2) and Lemma 3.7, we have \( \text{Jac}_f(x^*) N_1 \) is the zero vector. Note that the stoichiometric subspace \( S \) is spanned by the single vector \( N_1 \). So \( \text{im} (\text{Jac}_f(x^*)) \) is the subspace spanned by the zero vector, which is not equal to \( S \), and hence, this is a contradiction to the hypothesis that \( x^* \) is nondegenerate.
3.3. Relationship between multistationarity and multistability.

Theorem 3.14. Suppose $G \in \mathcal{G}$. If $\text{cap}_{\text{pos}}(G) = N \geq 2$ ($N \in \mathbb{Z}_{\geq 0}$), then $\text{cap}_{\text{stab}}(G) \leq \left\lceil \frac{N}{2} \right\rceil$.

Proof. The conclusion directly follows from Corollary 3.4, Lemma 3.2, Lemma 3.7, Theorem 3.5.

Theorem 3.15. Suppose $G \in \mathcal{G}_0$, or, suppose $G \in \mathcal{G}_1 \cup \mathcal{G}_2$ and $G$ has up to 2 species. If $\text{cap}_{\text{pos}}(G) = N \geq 2$ ($N \in \mathbb{Z}_{\geq 0}$), then $\left\lfloor \frac{N}{2} \right\rfloor \leq \text{cap}_{\text{stab}}(G) \leq \left\lceil \frac{N}{2} \right\rceil$.

Proof. The conclusion directly follows from Corollary 3.4, Lemma 3.2, Lemma 3.7, Theorem 3.5 and Theorem 3.12.

4. Networks in $\mathcal{G}_0$.

4.1. Boundary steady states and multistationarity.

Lemma 4.1. Given $G \in \mathcal{G}_0$, suppose the stoichiometric subspace of $G$ is one-dimensional. For the two systems $f(x)$ (3.3) and $h(x)$ (3.4), if $x^* \in \mathbb{R}^s$ is a solution to $f_1(x^*) = \ldots = f_s(x^*) = 0$, then

$$\text{(4.1) } |\text{Jac}_h(x^*)| = \kappa_1 (\alpha_{i1} - \beta_{i1})^{s-1} \prod_{k=1}^s x_k^{\alpha_{k1} - 1} \sum_{i=1}^s (\beta_{i1} - \alpha_{i1})(\alpha_{i1} - \alpha_{i2}) \prod_{k \neq i} x_k.$$

Proof. By (3.3), we have

$$\frac{\partial f_i}{\partial x_i} := \kappa_1 \alpha_{i1} (\beta_{i1} - \alpha_{i1}) x_i^{-1} \prod_{k=1}^s x_k^{\alpha_{k1}} - \lambda \kappa_2 \alpha_{i2} (\beta_{i1} - \alpha_{i1}) x_i^{-1} \prod_{k=1}^s x_k^{\alpha_{k2}},$$

and

$$\kappa_1 (\beta_{i1} - \alpha_{i1}) \prod_{k=1}^s x_k^{\alpha_{k1}} = \lambda \kappa_2 (\beta_{i1} - \alpha_{i1}) \prod_{k=1}^s x_k^{\alpha_{k2}}.$$

By (4.2) and (4.3), we have

$$\frac{\partial f_i}{\partial x_i}(x^*) := \kappa_1 (\beta_{i1} - \alpha_{i1})(\alpha_{i1} - \alpha_{i2}) x_i^{-1} \prod_{k=1}^s x_k^{\alpha_{k1}}.$$

Hence, by Lemma 3.7 and (4.4), we have (4.1).

Lemma 4.2. Given $G \in \mathcal{G}_0$, suppose the stoichiometric subspace of $G$ is one-dimensional. If $G$ admits a nondegenerate steady state, then the numbers in the sequence

$$\beta_{i1} - \alpha_{i1}, \ldots, (\beta_{s1} - \alpha_{s1})(\alpha_{s1} - \alpha_{s2})$$

cannot be all zeros.

Proof. The conclusion follows from Lemma 3.11 and Lemma 4.1.

Lemma 4.3. Given $G \in \mathcal{G}_0$, suppose the stoichiometric subspace of $G$ is one-dimensional. If the network $G$ admits multistationarity, then

$$\exists i, j \in \{1, \ldots, s\} \text{ s.t. } (\beta_{i1} - \alpha_{i1})(\alpha_{i1} - \alpha_{i2})(\beta_{j1} - \alpha_{j1})(\alpha_{j1} - \alpha_{j2}) < 0.$$
Thus, if the two monomials in $h$ by Lemma 4.7,

$$h \cap \Pi \leq 0$$

The two monomials $\Pi_{k=1}^{x_k^{\alpha_k}}$ and $\Pi_{k=1}^{x_k^{\alpha_k}}$ have no common variables).

**Proof.** Note that for $G \in \mathcal{G}_0$, we know $h_1$ defined in (3.4) is

$$(4.7) \quad h_1 = (\beta_{11} - \alpha_{11}) (\kappa_1 \Pi_{k=1}^{x_k^{\alpha_k_1}} - \lambda \kappa_2 \Pi_{k=1}^{x_k^{\alpha_k_2}}).$$

Clearly, if there exists $k \in \{1, \ldots, s\}$ such that $\alpha_k 1 > 0$ and $\alpha_k 2 > 0$, then there exists at least one boundary steady state $(x_1 = 0, \ldots, x_s = 0)$ in the stoichiometric compatibility class $P_c$ defined by $c = (0, \ldots, 0) \in \mathbb{R}^{s-1}$, which is a contradiction to the hypothesis that $G$ has no boundary steady state in any stoichiometric compatibility class.

**Example 4.6.** The converse of Lemma 4.5 might not be true. Consider the consistent network

$$X_1 + 2X_2 \xrightarrow{\kappa_1} X_2 + X_3, \quad X_3 \xrightarrow{\kappa_2} X_1 + X_2.$$  

The two monomials $\Pi_{k=1}^{x_k^{\alpha_k_1}} = x_1 x_2^1$ and $\Pi_{k=1}^{x_k^{\alpha_k_2}} = x_3$ have no common variables. For the total constants $c_1 = -1, c_2 = 1$, and for the rate constants $\kappa_1 = 25, \kappa_2 = 4$, there is a boundary steady state $(1, 0, 0)$.

**Lemma 4.7.** Given $G \in \mathcal{G}_0$, if for any $k (1 \leq k \leq s)$, we have either $\alpha_k 1 = 0$ or $\alpha_k 2 = 0$, then the network $G$ does not admit multistationarity.

**Proof.** Assume $G$ admits multistationarity. By Corollary 3.4, the stoichiometric subspace of $G$ is one-dimensional. For any $k (1 \leq k \leq s)$, we have either $\alpha_k 1 = 0$ or $\alpha_k 2 = 0$. If $\alpha_k 1 = 0$, then $(\beta_{k1} - \alpha_{k1})(\alpha_k 1 - \alpha_k 2) = -\beta_{k1} 2 \alpha_k 2 \leq 0$. If $\alpha_k 2 = 0$, then by Lemmas 3.3, there exists $\lambda > 0$ such that

$$(\beta_{k1} - \alpha_{k1})(\alpha_k 1 - \alpha_k 2) = -\frac{1}{\lambda}(\beta_{k1} - \alpha_{k1})(\alpha_k 1 - \alpha_k 2) = -\frac{1}{\lambda} \beta_{k1} 2 \alpha_k 1 \leq 0.$$

So, the signs of the non-zero numbers in the sequence (4.5) are all negative. By Lemma 4.3, $G$ does not admit multistationarity, which is a contradiction.

**Theorem 4.8.** Given $G \in \mathcal{G}_0$, if $G$ has no boundary steady state in any stoichiometric compatibility class, then the network $G$ does not admit multistationarity.

**Proof.** If $G$ has no boundary steady state in any stoichiometric compatibility class, by Lemma 4.5, for any $k (1 \leq k \leq s)$, we have either $\alpha_k 1 = 0$ or $\alpha_k 2 = 0$. By Lemma 4.7, $G$ does not admit multistationarity.

**4.2. Smallest multistable networks: proof of Theorem 2.5.**

**Theorem 4.9.** Given $G \in \mathcal{G}_0$, if $G$ is at-most-3-reactant, then $G$ does not admit multistability.

**Proof.** If the two monomials in $h_1 (x)$ (see (4.7)) have no common variables, then by Lemma 4.7, $G$ does not admit multistationarity. So, $G$ admits no multistability.

Note the total degree of $h_1 (x)$ w.r.t $x$ is at most 3 since $G$ is at-most-3-reactant. Thus, if the two monomials in $h_1 (x)$ have common variables, then the equations $h_1 (x) = \ldots = h_s (x) = 0$ have at most 2 common positive solutions. That means $cap_{pos} (G) \leq 2$. So, by Theorem 3.14, $cap_{stab} (G) \leq 1$. 

\[ \square \]
Lemma 4.10. Given $G \in G_0$, if $G$ has exactly 3 species and if $\text{cap}_{stab}(G) \geq 2$, then we have

\begin{align}
\beta_{k_1} - \alpha_{k_1} &\neq 0, \quad \text{and} \\
\alpha_{k_1} - \alpha_{k_2} &\neq 0, \quad \text{for any } k \in \{1, 2, 3\},
\end{align}

and we also have

\begin{align}
\frac{\beta_{j_2} - \alpha_{j_2}}{\beta_{j_1} - \alpha_{j_1}} &= \frac{\beta_{22} - \alpha_{22}}{\beta_{21} - \alpha_{21}} = \frac{\beta_{32} - \alpha_{32}}{\beta_{31} - \alpha_{31}} < 0.
\end{align}

Proof. Clearly, if $\text{cap}_{stab}(G) \geq 2$, then $\text{cap}_{nond}(G) \geq 2$ and $\text{cap}_{pos}(G) \geq 2$. So, by Corollary 3.4, the stoichiometric subspace of $G$ is one-dimensional, and hence the steady states are common solutions to the equations (4.8) and (4.15), with $x_0$ given by Corollary 3.4, the stoichiometric subspace of (4.15).

Note that by (3.4), there exist two distinct numbers $j_1, j_2 \in \{1, 2, 3\}$ such that for any $k \in \{j_1, j_2\}$, $(\beta_{k_1} - \alpha_{k_1})(\alpha_{k_1} - \alpha_{k_2}) \neq 0$. Without loss of generality, assume $j_1 = 1$ and $j_2 = 2$. Below, we show $(\beta_{j_1} - \alpha_{j_1})(\alpha_{j_1} - \alpha_{j_2}) \neq 0$.

In fact, we can rewrite the equations $h_1(x) = h_2(x) = h_3(x) = 0$ as

\begin{align}
x_2 &= \delta x_1^{-\xi_{12}} x_3^{-\xi_{32}} := \ell_1(x_1, x_3), \\
x_2 &= A_2 x_1 - B_2 := \ell_2(x_1), \\
x_3 &= A_3 x_1 - B_3 := \ell_3(x_1),
\end{align}

where $\delta = (\frac{\lambda_{k_1}}{\alpha_{k_1}})^{1 - \frac{\xi_{12}}{\xi_{32}}} > 0$, $\xi_{ij} = \frac{\alpha_{ij} - \alpha_{i}}{\alpha_{j} - \alpha_{ij}}$, $i, j = 1, 3$, $A_j = \frac{\beta_{j} - \alpha_{j}}{\beta_{j_1} - \alpha_{j_1}}$, and $B_j = \frac{\beta_{j} - \alpha_{j}}{\beta_{j_1} - \alpha_{j_1}}$.

If $\beta_{j_1} - \alpha_{j_1} = 0$, then the equation (4.13) becomes $x_3 = -B_3$, and so, the bivariate function $\ell_1(x_1, x_3)$ in (4.11) becomes $\ell_1(x_1) = \delta(-B_3)^{-\xi_{32}} x_1^{-\xi_{32}}$. Note that $\ell_1(x_1)$ is a linear function, and $\ell_2(x_1)$ is a power function. There are at most 2 intersection points of their graphs in the first quadrant. So the equations $h_1(x) = h_2(x) = h_3(x) = 0$ have at most 2 common positive solutions. By Theorem 3.14, $G$ admits no multistability, which is a contradiction. If $\alpha_{j_1} - \alpha_{j_2} = 0$, then $\ell(x_1, x_3)$ becomes a power function $\ell_1(x_1) = \delta x_1^{-\xi_{32}}$. With a similar argument, we can deduce a contradiction.

Finally, by Lemma 3.3, we have (4.10) since $\beta_{j_1} - \alpha_{j_1} \neq 0$ for all $k \in \{1, 2, 3\}$. 

For $k = 1, \ldots, s$, define $\gamma_k := \min\{\alpha_{k_1}, \alpha_{k_2}\}$, and $\alpha_{kj} := \alpha_{kj} - \gamma_k$ $(j = 1, 2)$. Then $h_1 (4.7)$ can be written as

\begin{align}
\tilde{h}_1 &= (\beta_{j_1} - \alpha_{j_1}) \left( \kappa_1 \Pi_{k=1}^s x_k^{\tilde{a}_{k_1}} - \lambda \kappa_2 \Pi_{k=1}^s x_k^{\tilde{a}_{k_2}} \right).
\end{align}

Let

\begin{align}
\tilde{g}(x) := \tilde{h}_1(x_1, \ldots, x_s) |_{x_2 = \frac{\beta_{j_1} - \alpha_{j_1}}{\beta_{j_1} - \alpha_{j_1}} x_1 - \frac{\gamma_1}{\alpha_{j_1} - \alpha_{j_1}}, \ldots, x_s = \frac{\beta_{j_1} - \alpha_{j_1}}{\beta_{j_1} - \alpha_{j_1}} x_1 - \frac{\gamma_s}{\alpha_{j_1} - \alpha_{j_1}}},
\end{align}

Lemma 4.11. Given $G \in G_0$, suppose the stoichiometric subspace of $G$ is one-dimensional. Let $g(x_1)$ and $\tilde{g}(x_1)$ be the polynomials respectively defined in (3.7) and (4.15). For a fixed rate-constant vector $\kappa^* \in \mathbb{R}^2_{\geq 0}$ and a total-constant vector $c^* \in \mathbb{R}^{s-1}$, if $G$ has a positive steady state $x^*$, then $g(x_1)$ has the same sign with

\begin{align}
\sum_{i=1}^s (\beta_{j_1} - \alpha_{j_1})(\alpha_{j_1} - \alpha_{j_2}) \Pi_{k \neq i} x_k^*.
\end{align}

and additionally, if $x^*$ is a stable positive steady state, then $\tilde{g}'(x_1^*) < 0$. 

Proof. Since the stoichiometric subspace of \( G \) is one-dimensional, the steady state \( x^* \) is a common solution to the equations \( h_1(x) = \ldots = h_n(x) = 0 \) (see (3.4)). By (4.15), we have \( \tilde{g}(x^*_1) = 0 \). So, comparing (3.7) and (4.15), we have \( \tilde{g}'(x^*_1) = \Pi_{k=1}^n \alpha_k^{-1} \tilde{g}'(x^*_1) \). By Lemma 3.8 and Lemma 4.1, \( \tilde{g}'(x^*_1) \) has the same sign with (4.16), and so, \( \tilde{g}'(x^*_1) \) has the same sign with (4.16). Additionally, if \( x^* \) is stable, by Lemma 3.2, Lemma 3.7 and Lemma 4.1, we know the sign of (4.16) is negative, and hence, \( \tilde{g}'(x^*_1) < 0 \).

**Lemma 4.12.** Given \( G \in \mathcal{G}_0 \), suppose the stoichiometric subspace of \( G \) is one-dimensional. Let \( I := (a, A) \) be the interval defined in (3.12), where \( a \in \mathbb{R} \), and \( A \in \mathbb{R} \cup \{ +\infty \} \). Let \( \tilde{g}(x_1) \) be the polynomial defined in (4.15). For a rate-constant vector \( \kappa^* \) and a total-constant vector \( c^* \), if the degree of \( \tilde{g}(x_1) \) w.r.t \( x_1 \) is 3, and if \( G \) has at least two stable positive steady states, then \( \tilde{g}(x_1) \) satisfies the three conditions below:

(i) \( \tilde{g}(a) > 0 \),

(ii) \( \tilde{g}(A) < 0 \) (here, \( \tilde{g}(+\infty) := \lim_{x_1 \to +\infty} \tilde{g}(x_1) \)), and

(iii) there exists \( x^*_1 \in (a, A) \) such that \( \tilde{g}(x^*_1) = 0 \) and \( \tilde{g}'(x^*_1) > 0 \).

Proof. By (4.15), we clearly see that if \( x^* \) is a positive steady state, then \( x^*_1 \in (a, A) \) and \( \tilde{g}(x^*_1) = 0 \). If \( x^* \) is stable, then by Lemma 4.11, we have \( \tilde{g}'(x^*_1) < 0 \). So, if \( G \) has at least two stable positive steady states \( x^{(1)} \) and \( x^{(2)} \) (here, we assume \( x^{(1)} < x^{(2)} \)), then for \( i \in \{1, 2\} \), \( x^{(i)} \in (a, A) \), \( \tilde{g}(x^{(i)}) = 0 \) and \( \tilde{g}'(x^{(i)}) < 0 \). Since the degree of \( \tilde{g}(x_1) \) w.r.t \( x_1 \) is 3, there exists a third simple real root \( x^*_1 \) to the equation \( \tilde{g}(x_1) = 0 \). By Lemma 3.6, we know \( x^*_1 \in (x^{(1)}, x^{(2)}) \subset (a, A) \), and \( \tilde{g}'(x^*_1) > 0 \) (i.e., the statement (iii) is proved). We can write \( \tilde{g}(x_1) \) as

\[
\tilde{g}(x_1) = C(x_1 - x^{(1)})(x_1 - x^{(1)})(x_1 - x^{(2)}), \quad \text{where } C \in \mathbb{R}.
\]

So \( \tilde{g}'(x^{(1)}) < 0 \) implies that \( \tilde{g}'(x^{(1)}) = C(x^{(1)} - x^{(1)})(x^{(1)} - x^{(2)}) < 0 \) (i.e., \( C < 0 \)).

Thus, \( \tilde{g}(a) = C(a - x^{(1)})(a - x^{(1)})(a - x^{(2)}) > 0 \) (the statement (i)). Similarly, it is directly to see \( \tilde{g}(A) < 0 \) (the statement (ii)).

**Definition 4.13.** Given matrices of reactant coefficients \( \alpha = (\alpha_{kj})_{s \times 2} \) and \( \hat{\alpha} = (\hat{\alpha}_{kj})_{s \times 2} \), which are associated with two networks \( G \) and \( \hat{G} \) in \( \mathcal{G}_0 \), we say \( \alpha \) is equivalent to \( \hat{\alpha} \), if there exist finitely many matrices \( \alpha^{(0)}, \ldots, \alpha^{(n)} \) such that \( \alpha^{(0)} = \alpha \), \( \alpha^{(n)} = \hat{\alpha} \), and for any \( i \in \{0, \ldots, n-1\} \), we can obtain \( \alpha^{(i+1)} \) from \( \alpha^{(i)} \) by switching two rows or two columns of \( \alpha^{(i)} \).

Clearly, if a network \( \hat{G} \in \mathcal{G}_0 \) has the form of a network \( G \in \mathcal{G}_0 \), then the two matrices of reactant coefficients associated with \( G \) and \( \hat{G} \) are equivalent (remark that the converse might not be true). Recall Example 2.2. The two sets of reactant coefficients (say \( \alpha \) and \( \hat{\alpha} \) of networks (2.6) and (2.7) can be written as matrices

\[
\alpha = \begin{pmatrix} 1 & 0 \\ 0 & 1 \\ 1 & 2 \end{pmatrix} \quad \text{and} \quad \hat{\alpha} = \begin{pmatrix} 0 & 1 \\ 2 & 1 \\ 1 & 0 \end{pmatrix}.
\]

We can obtain \( \alpha \) from \( \hat{\alpha} \) by first switching the two columns and then switching the last two rows.

**Lemma 4.14.** If a 3-species network \( G \in \mathcal{G}_0 \) is at-most-4-reactant, and if \( G \) admits multistability, then \( G \) can only have the form of one of the networks listed in Table 2.

Proof. If \( G \) admits multistability, then by Theorem 4.9, \( G \) must be 4-reactant,
and so, the degree of \( h_1 \) \( w.r.t \) \( x \) is exactly 4, i.e.,

\[
\text{(4.17)} \quad \max \{ \sum_{k=1}^{3} \alpha_{k1}, \sum_{k=1}^{3} \alpha_{k2} \} = 4.
\]

By Lemma 4.7, the two monomials in \( h_1 \) have common variables. So, the degree of \( \hat{h}_1 \) \( w.r.t \) \( x \) is at most 3. On the other hand, Theorem 3.14 implies that if \( G \) admits multistability, then \( \text{cap}_{pos}(G) \geq 3 \). Note that all positive steady states of \( G \) are common solutions to the equations \( \hat{h}_1(x) = h_2(x) = \ldots = h_s(x) = 0 \). So, the degree of \( \hat{h}_1 \) \( w.r.t \) \( x \) is at least 3. Overall, the degree of \( \hat{h}_1 \) \( w.r.t \) \( x \) is exactly 3. So, by the definition of \( \hat{h}_1 \), we have

\[
\text{(4.18)} \quad \sum_{k=1}^{3} \min \{ \alpha_{k1}, \alpha_{k2} \} = 1.
\]

Therefore, by Lemma 4.3 and Lemma 4.10, we know that the matrix of reactant coefficients \( \alpha := (\alpha_{kj})_{3 \times 2} \) and the matrix of product coefficients \( \beta := (\beta_{kj})_{3 \times 2} \) associated with \( G \) belong to the set

\[
\text{(4.19)} \quad B := \{ (\alpha, \beta) \in \mathbb{Z}_{\geq 0}^{3 \times 2} \times \mathbb{Z}_{\geq 0}^{3 \times 2} \text{ s.t. (4.6), (4.9), (4.10), (4.17) and (4.18) hold} \}.
\]

Define a map \( \pi : \mathbb{Z}_{\geq 0}^{3 \times 2} \times \mathbb{Z}_{\geq 0}^{3 \times 2} \to \mathbb{Z}_{\geq 0}^{3 \times 2} \) such that for any \( (\alpha, \beta) \in \mathbb{Z}_{\geq 0}^{3 \times 2} \times \mathbb{Z}_{\geq 0}^{3 \times 2} \), \( \pi(\alpha, \beta) = \alpha \). Let

\[
\text{(4.20)} \quad B_{\alpha} := \{ \alpha \in \mathbb{Z}_{\geq 0}^{3 \times 2} \text{ s.t. (4.9), (4.17) and (4.18) hold} \}.
\]

Notice that \( B_{\alpha} \) is a finite set. For each \( \alpha \in B_{\alpha} \), define its equivalence class in \( B_{\alpha} \) as \([\alpha] := \{ \hat{\alpha} \in B_{\alpha} | \hat{\alpha} \text{ is equivalent to } \alpha \}\). We explicitly compute the set \( B_{\alpha} \) by Maple2020 [15], and it is straightforward to check by a computer program that there are 12 equivalence classes in \( B_{\alpha} \) (see a supporting file “irreversible.mw” in Table 5). We pick up a representative from each equivalence class, and we present them in Table 1.

Note

\[
\text{(4.21)} \quad B = \pi^{-1}(B_{\alpha}) \cap B = \pi^{-1}(\cup_{\alpha \in B_{\alpha}} [\alpha]) \cap B = \cup_{\alpha \in B_{\alpha}} \cup_{\hat{\alpha} \in [\alpha]} (\pi^{-1}(\hat{\alpha}) \cap B).
\]

By Definition 4.13, if \( \hat{\alpha} \in [\alpha] \), then there exist two permutation matrices \( P \) and \( Q \) such that \( \hat{\alpha} = P \alpha Q \). Thus, there exists a bijection \( \phi : \pi^{-1}(\alpha) \cap B \to \pi^{-1}(\hat{\alpha}) \cap B \) such that for any \( (\alpha, \beta) \in \pi^{-1}(\alpha) \cap B \), \( \phi(\alpha, \beta) := (\hat{\alpha}, P \beta Q) \). By Definition 2.1, the two networks associated with \( (\alpha, \beta) \) and \( \phi(\alpha, \beta) \) have the same form. Thus, by (4.21), the multistable network \( G \) has the form of a network associated with an element in \( \pi^{-1}(\alpha) \cap B \) for a representative \( \alpha \) in \( B_{\alpha} \). In the rest of the proof, we explain how to compute \( \pi^{-1}(\alpha) \cap B \) for each representative recorded in Table 1.

For the values of \( \alpha_{kj} \) recorded in Table 1-Row (1), the condition (4.10) implies

\[
\text{(4.22)} \quad (\beta_{12} - 1)/(\beta_{11} - 2) < 0,
\]
\[
\text{(4.23)} \quad \beta_{22}/(\beta_{21} - 1) < 0,
\]
\[
\text{(4.24)} \quad \beta_{32}/(\beta_{31} - 1) < 0.
\]

Note that \( \beta_{kj} \in \mathbb{Z}_{\geq 0} \). So by (4.23) and (4.24), we have \( \beta_{21} = \beta_{31} = 0 \). Also, note that the sequence (4.5) is now

\[
\text{(4.25)} \quad \beta_{11} - 2, \beta_{21} - 1, \text{ and } \beta_{31} - 1.
\]
Similarly, from each _h_ it is straightforward to check by Lemma 3.2 that the equality (3.1) holds for _ξ_ = 1, _κ_ = 9, _c_ = 6, and _c_ = -9. By solving the equations _h_1(1) = _h_2(1) = _h_3(1) = 0 (see (3.4) and (4.7)), we see that the network has three nondegenerate positive steady states:

\[ x^{(1)} = \left( \frac{7}{2} + \frac{\sqrt{205}}{6}, \frac{5}{2} + \frac{\sqrt{205}}{6}, \frac{12}{5} + \frac{\sqrt{205}}{6} \right), \quad x^{(2)} = (5, 1, \frac{9}{10}), \quad x^{(3)} = (\frac{7}{2} + \frac{\sqrt{205}}{6}, \frac{5}{2} - \frac{\sqrt{205}}{6}, \frac{12}{5} - \frac{\sqrt{205}}{6}). \]

It is straightforward to check by Lemma 3.2 that _x^{(1)}_ and _x^{(3)}_ are stable.

For the network (2.9), if _β_21 = 0, then for any _β_12 _∈_ _Z_ > 0, _β_22 = 2_β_12 and _β_32 = _β_12 + 2. It is straightforward to check that the equality (3.1) holds for _ξ_ = _β_12 > 0. Let _κ_1 = 1, _κ_2 = 48, _c_1 = \frac{12}{2}, and _c_2 = \frac{1}{4}. Then we have

\[ h_1 = (\beta_{11} - \alpha_{11}) (\kappa_1 \Pi_{k=1}^{\omega_{k1}} - \lambda_2 \Pi_{k=1}^{\omega_{k2}}) = -\left( x_1^2 x_2 x_3 - 48 x_3^3 \right), \]
\[ h_2 = (\beta_{11} - \alpha_{11}) x_1 - (\beta_{11} - \alpha_{11}) x_2 - c_1 = -2 x_1 + x_2 - \frac{13}{2}, \quad \text{and} \]
\[ h_3 = (\beta_{31} - \alpha_{31}) x_1 - (\beta_{11} - \alpha_{11}) x_3 - c_2 = -x_1 + x_3 - \frac{1}{4}. \]

By solving the equations _h_1(1) = _h_2(1) = _h_3(1) = 0, the network has three nondegenerate positive steady states:

\[ x^{(1)} = \left( \frac{19}{8} - \frac{3\sqrt{33}}{4}, \frac{45}{8}, \frac{21}{8} - \frac{3\sqrt{33}}{8} \right), \quad x^{(2)} = \left( \frac{3}{4}, 8, 1 \right), \quad x^{(3)} = \left( \frac{19}{8} + \frac{3\sqrt{33}}{4}, \frac{45}{8} + \frac{3\sqrt{33}}{8}, \frac{21}{8} + \frac{3\sqrt{33}}{8} \right). \]

It is straightforward to check by Lemma 3.2 that _x^{(1)}_ and _x^{(3)}_ are stable. Similarly, if _β_21 = 1, then for any _β_12 _∈_ _Z_ > 0, _β_22 = _β_12 and _β_32 = _β_12 + 2. Let _κ_1 = 1, _κ_2 = \frac{12}{b_2},
\[ c_1 = \frac{13}{4}, \text{ and } c_2 = \frac{1}{4}. \] Then the network has three nondegenerate positive steady states:

\[ x^{(1)} = \left( \frac{19}{8} - 3\sqrt{\frac{3}{8}}, \frac{45}{8} - 3\sqrt{\frac{3}{8}}, \frac{21}{8} - 3\sqrt{\frac{3}{8}} \right), x^{(2)} = \left( \frac{3}{4}, 4, 1 \right), x^{(3)} = \left( \frac{19}{8} + 3\sqrt{\frac{3}{8}}, \frac{45}{8} + 3\sqrt{\frac{3}{8}}, \frac{21}{8} + 3\sqrt{\frac{3}{8}} \right). \]

It is straightforward to check by Lemma 3.2 that \( x^{(1)} \) and \( x^{(3)} \) are stable. Here, we compute these steady states by Maple2020 [15], see “witness1.mw” in Table 5.

“⇒”: By Theorem 3.15 and [13, Theorem 3.6 2(b), Theorem 4.8], if \( G \in G_0 \) and \( G \) has up to 2 species, then \( G \) admits no multistability. From Table 2, it is directly seen that the networks (2.8) and (2.9) are respectively listed in Row (3) and Row (7). By Lemma 4.14, we only need to show none of the other networks listed in Table 2 admits multistability.

For the network in Table 2-Row (1), the polynomial \( \tilde{g}(x_1) \) defined in (4.15) is

\[ \tilde{g}(x_1) = \kappa_1 x_1 x_2 x_3 - \lambda \kappa_2 \mid x_2 = -x_1 - c_1, x_3 = -x_1 - c_2, \]

where \( \lambda := -\frac{\beta_{12} - \alpha_{12}}{\beta_{11} - \alpha_{11}} > 0 \), and the interval \( I \) defined in (3.12) is \( (0, \min\{-c_1, -c_2\}) \). Note that \( \tilde{g}(0) = -\lambda \kappa_2 < 0 \) for any \( \kappa_2 \in \mathbb{R}_{>0} \). So, by Lemma 4.12, this network in Row (1) does not admit multistability.

For the network in Table 2-Row (2), the polynomial \( \tilde{g}(x_1) \) is

\[ \tilde{g}(x_1) = \kappa_1 x_1 x_2^2 - \lambda \kappa_2 x_3 \mid x_2 = \frac{\beta_{21} - 2}{\beta_{11} - 2} x_1 - \frac{c_1}{\beta_{11} - 2}, x_3 = \frac{\beta_{11} - 2}{\beta_{11} - 2} x_1 - \frac{c_2}{\beta_{11} - 2}, \]

where \( \lambda := -\frac{\beta_{12} - \alpha_{12}}{\beta_{11} - \alpha_{11}} > 0 \), and the interval \( I \) is \( (\max\{0, \frac{c_1}{\beta_{11} - 2}\}, -\frac{c_1}{\beta_{11} - 2}) \). From the second column of Row (2), we see that \( \beta_{11} - 2 > 0 \), \( \beta_{21} - 2 = -\beta_{22}(\beta_{11} - 2) < 0 \), and \( \beta_{31} = \beta_{11} - 2 > 0 \). If \( \frac{c_2}{\beta_{31}} < 0 \), then \( \tilde{g}(0) = \lambda \kappa_2 \frac{c_2}{\beta_{31} - 2} < 0 \), and so, by Lemma 4.12 (i), the network in Row (2) does not admit multistability. If \( \frac{c_2}{\beta_{31}} \geq 0 \), then by Lemma 4.11, for any positive steady state \( x^* \) of \( G \), \( \tilde{g}(x_1^*) \) has the same sign with

\[ \sum_{i=1}^{3} (\beta_{11} - \alpha_{11})(\alpha_{11} - \alpha_{12}) \Pi_k \neq i x_k^* \]

\[ = (\beta_{11} - 2)x_2^* x_3^2 + 2(\beta_{21} - 2)x_1^* x_3^2 - \beta_{31} x_1^* x_3^2 \]

\[ = (\beta_{11} - 2)x_3^* - \beta_{31} x_1^* x_3^2 + 2(\beta_{21} - 2)x_1^* x_3^2 \]

\[ = -c_2 x_3^* + 2(\beta_{21} - 2)x_1^* x_3^2, \]

which is negative (Note \( \beta_{21} - 2 < 0 \)). So by Lemma 4.12 (iii), the network in Row (2) does not admit multistability. Similarly, we can prove the networks in Rows (5), (8), and (12) do not admit multistability.

For the network in Table 2-Row (4), the polynomial \( \tilde{g}(x_1) \) is

\[ \tilde{g}(x_1) = -\left( \kappa_1 x_1 x_2^2 - \lambda \kappa_2 x_3 \right) \mid x_2 = -(\beta_{21} - 2)x_1 + c_1, x_3 = x_1 + c_2, \]

where \( \lambda := -\frac{\beta_{12} - \alpha_{12}}{\beta_{11} - \alpha_{11}} > 0 \), and the interval \( I \) is \( (\max\{0, \frac{c_1}{\beta_{21} - 2}, -c_2\}, +\infty) \). From the second column of Row (4), we see that \( \beta_{21} - 2 < 0 \) If \( -c_2 > 0 \) and \(-c_2 < \frac{c_1}{\beta_{21} - 2} \), then by the fact that \( \tilde{g}(-c_2) = \kappa_1 c_2 ((\beta_{21} - 2) c_2 + c_1)^2 \leq 0 \) and by Lemma 4.12 (i), the network in Row (4) does not admit multistability. If \(-c_2 \leq 0 \), then by Lemma
where from the two conservation law equations multistability, then the network in Row (11) does not admit multistability. 

\[ \lambda \kappa \]

Similarly, if \(-c_2 \leq \frac{c_1}{\beta_{31} - \beta_{32}}\) (i.e., \(c_1 + c_2(\beta_{31} - 2) \leq 0\)), then we also have

\[ \sum_{i=1}^{3} (\beta_{i1} - \alpha_{i1})(\alpha_{i1} - \alpha_{i2}) \Pi_{k \neq i} x_k^* \]

Similarly, if \(-c_2 \leq \frac{c_1}{\beta_{31} - \beta_{32}}\) (i.e., \(c_1 + c_2(\beta_{31} - 2) \leq 0\)), then we also have

\[ \sum_{i=1}^{3} (\beta_{i1} - \alpha_{i1})(\alpha_{i1} - \alpha_{i2}) \Pi_{k \neq i} x_k^* \]

\[ = -x_2^* x_3^* + 2(\beta_{21} - 2)x_1^* x_3^* + x_2^* x_2^* \]

\[ = -x_2^* x_3^* + (\beta_{21} - 2)x_1^* x_3^* + x_1^* (\beta_{21} - 2)x_3^* + x_2^* \]

\[ = -x_2^* x_3^* + (\beta_{21} - 2)x_1^* x_3^* + x_1^* (c_1 + c_2(\beta_{21} - 2)) < 0 \]

(note the last equality \((\beta_{21} - 2)x_1^* + x_2^* = c_1 + c_2(\beta_{21} - 2)\) above is deduced by eliminating \(x_1^*\) from the two conservation law equations \((\beta_{21} - 2)x_1^* + x_2^* - c_1 = 0\) and \(-x_1^* + x_3^* - c_2 = 0\). So by Lemma 4.12 (iii), the network in Row (4) does not admit multistability. Similarly, we can prove the network in Row (10) does not admit multistability.

For the network in Table 2-Row (6), the polynomial \(\tilde{g}(x_1)\) is

\[ \tilde{g}(x_1) = -\left( \kappa_1 x_1 x_2^2 - \lambda \kappa_2 x_3^2 \right) \bigg|_{x_2 = -x_1 + c_1, x_3 = -\beta_{31}x_1 + c_2} \]

where \(\lambda := \frac{-\beta_{32} - \alpha_{i2}}{\beta_{31} - \alpha_{i1}} > 0\), and the interval \(I\) is \((0, \min\{c_1, \frac{c_1}{\beta_{32}}\})\) (from the second column of Row (4), we see that \(\lambda < 0\)). If \(c_1 < \frac{c_1}{\beta_{31}}\), then by the fact that \(\tilde{g}(c_1) = \lambda \kappa_2(-\beta_{31}c_1 + c_2)^2 \geq 0\) and by Lemma 4.12 (ii), the network in Row (2) does not admit multistability. If \(\frac{c_1}{\beta_{32}} \leq c_1\) (i.e., \(-\beta_{31}c_1 + c_2 \leq 0\)), then by Lemma 4.11, for any positive steady state \(x^*\) of \(G\), \(\tilde{g}(x_1^*)\) has the same sign with

\[ \sum_{i=1}^{3} (\beta_{i1} - \alpha_{i1})(\alpha_{i1} - \alpha_{i2}) \Pi_{k \neq i} x_k^* \]

\[ = -x_2^* x_3^* + 2x_1^* x_3^* - 2\beta_{31} x_1^* x_2^* \]

\[ = -x_2^* x_3^* + 2x_1^* (x_3^* - \beta_{31} x_2^*) \]

\[ = -x_2^* x_3^* + 2x_1^* (-\beta_{31}c_1 + c_2) < 0 \]

(note the last equality \(x_3^* - \beta_{31} x_2^* = -\beta_{31}c_1 + c_2 \) above is deduced by eliminating \(x_1^*\) from the two conservation law equations \(x_1^* + x_2^* - c_1 = 0\) and \(\beta_{31}x_1^* + x_2^* - c_2 = 0\). So by Lemma 4.12 (iii), the network in Row (6) does not admit multistability. Similarly, the network in Row (9) does not admit multistability.

For the network in Table 2-Row (11), the polynomial \(\tilde{g}(x_1)\) is

\[ \tilde{g}(x_1) = (\beta_{11} - 4) \left( \kappa_1 x_1^3 - \lambda \kappa_2 x_2 x_3 \right) \bigg|_{x_2 = -\beta_{31} x_1, x_3 = -\frac{c_1}{\beta_{31}}, x_3 = -\frac{c_2}{\beta_{31}}} \]

where \(\lambda := -\frac{\beta_{32} - \alpha_{i2}}{\beta_{31} - \alpha_{i1}} > 0\), and the interval \(I\) is \((\max\{0, \frac{c_1}{\beta_{32}}, \frac{c_2}{\beta_{31}}\}, +\infty)\). Note that \(\tilde{g}(+\infty)\) has a positive sign for any \(\kappa_2 \in \mathbb{R}_{>0}\). So, by Lemma 4.12 (ii), if \(G\) admits multistability, then the network in Row (11) does not admit multistability. □
Remark 4.15. It is stated in [13, Remark 5.4] that the network recorded in Table 2—Row (1) admits multistability. But here, in the proof of Theorem 2.5, we proved it does not.

5. Networks in $\mathcal{G}_1$: proof of Theorem 2.6.

Lemma 5.1. [20, Theorem 3.5] Given $G \in \mathcal{G}_1$, if $G$ has exactly 2 species, then $G$ admits nondegenerate multistationarity if and only if there exists $\lambda \in \mathbb{R}\{0\}$ such that the equality (3.1) holds for $s = 2$, and

$$(5.1) \quad \exists k \in \{1, 2\} \text{ s.t. } \max\{\alpha_k, \beta_k\} < \alpha_k < \beta_k \text{ or } \min\{\alpha_k, \beta_k\} > \alpha_k > \beta_k.$$ 

Lemma 5.2. Suppose $G \in \mathcal{G}_1$, and suppose $G$ has exactly 2 species. If $G$ admits multistability, then we have

$$(5.2) \quad (\beta_{11} - \alpha_{11})(\beta_{21} - \alpha_{21}) \neq 0,$$

and

$$(5.3) \quad \frac{\beta_{12} - \alpha_{12}}{\beta_{11} - \alpha_{11}} = \frac{\beta_{22} - \alpha_{22}}{\beta_{21} - \alpha_{21}} \neq 0.$$ 

Proof. Recall that we have $\beta_{11} - \alpha_{11} \neq 0$ by Assumption 3.1. If $\beta_{21} - \alpha_{21} = 0$, then by (3.7), we have $g(x_1) = (\delta_{11} - \alpha_{11}) \left( \kappa_1 \Gamma^2 + \kappa_2 \Gamma \beta_{21} x_1^{\alpha_{11} - 1} - \kappa_3 \Gamma \beta_{21} x_1^{\alpha_{11} - 1} + \lambda_3 \Gamma^2 x_1^{\alpha_{11} - 1} \right)$, where $\Gamma = \frac{(\delta_{11} - \alpha_{11})}{(\beta_{11} - \alpha_{11})}$. So, $g(x_1) = 0$ has at most 3 terms, and hence, the number of sign changes of the coefficients is at most 2. By Descartes’ rule of signs [10] (see a supporting file “SM.pdf” in Table 5), $g(x_1) = 0$ has at most 2 positive roots. So, the
network $G$ admits at most 2 positive steady states. By Theorem 3.14, the network does not admit multistability, which is a contradiction. So, the inequality (5.2) holds. Finally, by Lemma 3.3, we have (5.3).

**DEFINITION 5.3.** Given two matrices of reactant coefficients

$$
\sigma = \begin{pmatrix}
\alpha_{11} & \beta_{11} & \alpha_{12} \\
\alpha_{21} & \beta_{21} & \alpha_{22}
\end{pmatrix}
$$

and

$$
\hat{\sigma} = \begin{pmatrix}
\hat{\alpha}_{11} & \hat{\beta}_{11} & \hat{\alpha}_{12} \\
\hat{\alpha}_{21} & \hat{\beta}_{21} & \hat{\alpha}_{22}
\end{pmatrix},
$$

which are associated with two 2-species networks $G$ and $\hat{G}$ in $\mathcal{G}_1$, we say $\sigma$ is strongly equivalent to $\hat{\sigma}$, if there exist finitely many matrices $\sigma^{(0)}, \ldots, \sigma^{(n)}$ such that $\sigma^{(0)} = \sigma$, $\sigma^{(n)} = \hat{\sigma}$, and for any $i \in \{0, \ldots, n-1\}$, we can obtain $\sigma^{(i+1)}$ from $\sigma^{(i)}$ by switching the two rows or the first two columns of $\sigma^{(i)}$.

**EXAMPLE 5.4.** Consider the two networks below.

(5.4) \[ X_1 + 2X_2 \iff 0, \quad 2X_1 \rightarrow 3X_1 + 2X_2. \]

(5.5) \[ 0 \iff 2X_1 + X_2, \quad 2X_2 \rightarrow 2X_1 + 3X_2. \]

The two matrices of reactant coefficients of networks (5.4) and (5.5) can be rewritten as

$$
\sigma = \begin{pmatrix}
1 & 0 & 0 \\
2 & 0 & 2
\end{pmatrix}
$$

and

$$
\hat{\sigma} = \begin{pmatrix}
0 & 2 & 2 \\
0 & 1 & 0
\end{pmatrix}.
$$

We can obtain $\sigma$ from $\hat{\sigma}$ by first switching the first two columns and then switching the two rows. So $\sigma$ is strongly equivalent to $\hat{\sigma}$.

**LEMMA 5.5.** If a 2-species network $G \in \mathcal{G}_1$ is at-most-3-reactant, and if $G$ admits multistability, then $G$ can only have the form of one of the networks listed in Table 4.

**Proof.** If $G$ admits multistability, then by Theorem 3.14, we have $\text{cap}_{\text{pos}}(G) \geq 3$. Note that all positive steady states of $G$ are common solutions to the equations $h_1(x) = \ldots = h_s(x) = 0$ (see (3.4)). So, the degree of $h_1 \ w.r.t \ x$ is at least 3. Since $G$ is at most 3-reactant, the degree of $h_1 \ w.r.t \ x$ is at most 3. Overall, the degree $h_1 \ w.r.t \ x$ is exactly 3, i.e.,

$$
\max\left\{\sum_{k=1}^{2} \alpha_{k1}, \sum_{k=1}^{2} \beta_{k1}, \sum_{k=1}^{2} \alpha_{k2}\right\} = 3.
$$

So, we have $\text{cap}_{\text{pos}}(G) = 3$. That means $G$ has no boundary steady states and so,

$$
\min\{\alpha_{11}, \beta_{11}, \alpha_{21}\} = 0, \quad \text{and} \quad \min\{\alpha_{21}, \beta_{21}, \alpha_{22}\} = 0.
$$
Therefore, by Lemma 5.1 and Lemma 5.2, we know that the matrix of reactant coefficients and product coefficients \( \tau := \begin{pmatrix} \alpha_{11} & \beta_{11} & \alpha_{12} & \beta_{12} \\
\alpha_{21} & \beta_{21} & \alpha_{22} & \beta_{22} \end{pmatrix} \) associated with \( G \) belong to the set
\[
(5.8) \quad C := \{ \begin{pmatrix} \alpha_{11} & \beta_{11} & \alpha_{12} & \beta_{12} \\
\alpha_{21} & \beta_{21} & \alpha_{22} & \beta_{22} \end{pmatrix} \in \mathbb{Z}_{\geq 0}^{2 \times 4} \text{ s.t. } (5.1), (5.2), (5.3), (5.6), (5.7) \text{ hold} \}
\]

Define a map \( \pi_\sigma : \mathbb{Z}_{\geq 0}^{2 \times 4} \rightarrow \mathbb{Z}_{\geq 0}^{2 \times 3} \) such that for any \( \begin{pmatrix} \alpha_{11} & \beta_{11} & \alpha_{12} & \beta_{12} \\
\alpha_{21} & \beta_{21} & \alpha_{22} & \beta_{22} \end{pmatrix} \in \mathbb{Z}_{\geq 0}^{2 \times 4} \), its image under \( \pi_\sigma \) is \( \begin{pmatrix} \alpha_{11} & \beta_{11} & \alpha_{12} \\
\alpha_{21} & \beta_{21} & \alpha_{22} \end{pmatrix} \). Note that if \( \tau \) satisfies (5.1), then \( \pi_\sigma(\tau) \) satisfies
\[
(5.9) \quad \exists k \in \{1, 2\} \text{ s.t. } \max\{\alpha_{k1}, \beta_{k1}\} < \alpha_{k2}, \text{ or } \min\{\alpha_{k1}, \beta_{k1}\} > \alpha_{k2} > 0.
\]

Let
\[
(5.10) \quad C_\sigma := \{ \begin{pmatrix} \alpha_{11} & \beta_{11} & \alpha_{12} \\
\alpha_{21} & \beta_{21} & \alpha_{22} \end{pmatrix} \in \mathbb{Z}_{\geq 0}^{2 \times 3} \text{ s.t. } (5.2), (5.6), (5.7) \text{ hold} \}
\]

For any \( \sigma \in C_\sigma \), define an equivalence class \( C_\sigma \) as
\[
[\sigma]_C := \{ \hat{\sigma} \in C_\sigma | \hat{\sigma} \text{ is strongly equivalent to } \sigma \}.
\]

It is straightforward to check by a computer program that there are 25 equivalence classes in \( C_\sigma \) (see supporting files “reverse1.mw”–“reverse4.mw” in Table 5), and we pick up one element from each equivalence class as a representative. We present the 25 representatives in Table 3.

In Table 3, for any representative \( \sigma \) recorded in a unbold/uncolored cell, the set \( \pi^{-1}_\sigma(\sigma) \cap C \) is empty. For instance, for the first column of the second row, we have
\[
\sigma = \begin{pmatrix} \alpha_{11} & \beta_{11} & \alpha_{12} \\
\alpha_{21} & \beta_{21} & \alpha_{22} \end{pmatrix} = \begin{pmatrix} 0 & 1 & 2 \\
3 & 0 & 1 \end{pmatrix},
\]

which satisfies the condition (5.9) because for \( k = 1 \), \( \max\{\alpha_{k1}, \beta_{k1}\} < \alpha_{k2} \) holds. By the condition (5.1), we have \( \beta_{12} > \alpha_{12} = 2 \). The condition (5.3) can be written as \( \beta_{22} - \beta_{12} = \beta_{22} - 1 = 1 \). So, we have \( \beta_{22} - 1 < 0 \) since \( \beta_{12} - 2 > 0 \). Hence, \( \beta_{22} = 0 \) is the only solution for \( \beta_{22} \) in \( \mathbb{Z}_{\geq 0} \). So we have \( \beta_{12} - 2 = \frac{1}{3} \), and we have no solution for \( \beta_{12} \) in \( \mathbb{Z}_{\geq 0} \). Therefore, \( \pi^{-1}_\sigma(\sigma) \cap C = \emptyset \). Similarly, we can easily verify that \( \pi^{-1}_\sigma(\sigma) \cap C \) is empty for any other \( \sigma \) recorded in a unbold cell (see “reverse1.mw”–“reverse4.mw”). We repeat the representatives in the bold/color cells in the first column of Table 4, and we write down their corresponding networks in the second column.

Note
\[
(5.11) \quad \mathcal{C} = \pi^{-1}_\sigma(\sigma) \cap C = \cup_{\sigma \in C_\sigma} \cup_{\hat{\sigma} \in [\sigma]_C} \left( \pi^{-1}_\sigma(\hat{\sigma}) \cap C \right).
\]

By Definition 5.3, if \( \hat{\sigma} \in [\sigma]_C \), then there exist two permutation matrices \( P \) and \( Q \) such that \( \hat{\sigma} = PrQ \). Thus, there exists a bijection \( \phi : \pi^{-1}_\sigma(\sigma) \cap C \rightarrow \pi^{-1}_\sigma(\hat{\sigma}) \cap C \) such that for any \( \tau \in \pi^{-1}_\sigma(\sigma) \cap C \), \( \phi(\tau) := PrQ \). By Definition 2.1, the two networks associated with \( \tau \) and \( \phi(\tau) \) have the same form. Thus, by (5.11), any multistable network \( G \) has the form of a network associated with an element in \( \pi^{-1}_\sigma(\sigma) \cap C \) for a representative \( \sigma \) recorded in the first column of Table 4. In the rest of the proof, we explain how to compute \( \pi^{-1}_\sigma(\sigma) \cap C \) for each representative in \( C_\sigma \) recorded in Table 4.
For the reactant coefficients recorded in Table 4-Row (1), the matrix \( \sigma \) is

\[
\begin{pmatrix}
\alpha_{11} & \beta_{11} & \alpha_{12} \\
\alpha_{21} & \beta_{21} & \alpha_{22}
\end{pmatrix} = \begin{pmatrix}
2 & 0 & 3 \\
1 & 0 & 0
\end{pmatrix},
\]

which satisfies the condition (5.9) because for \( k = 1 \), \( \max\{\alpha_{k1}, \beta_{k1}\} < \alpha_{k2} \) holds. By the condition (5.1), we have \( \beta_{12} > \alpha_{12} = 3 \). The condition (5.3) can be written as \( \frac{\beta_{12} - 3}{2} = \frac{\beta_{22}}{2} \), i.e., \( \beta_{22} = \frac{1}{2}(\beta_{12} - 3) \). Above all, we conclude that

\[
\pi_\sigma^{-1}(\sigma) \cap \mathcal{C} = \left\{ \left( \begin{array}{cccc}
2 & 0 & 3 \\
1 & 0 & 0
\end{array} \right) \beta_{12} \in \mathbb{Z}_{>3} \right\}.
\]

Similarly, from each set of reactant coefficients recorded in the first column of Table 4, we can solve \( \pi_\sigma^{-1}(\sigma) \cap \mathcal{C} \), and we record the corresponding \( \beta_{21} \) and \( \beta_{22} \) in the third column.

**Proof of Theorem 2.6.**  “\( \Leftarrow \): For the network in Table 4–Row (7), it is straightforward to check that for any \( \beta_{12} > 2 \), the equality (3.1) holds for \( \lambda = -\beta_{12} - 2 < 0 \). Let \( \kappa_1 = \frac{1}{2}, \kappa_2 = 16, \kappa_3 = \frac{3}{2(\beta_{12} - 2)} \) and \( c_1 = -9 \). Then we have

\[
h_1 = (\beta_{11} - \alpha_{11})(\kappa_1 x_2 - \kappa_2 x_1 - \lambda \kappa_3 x_2^2 x_2) = \frac{1}{2} x_2 - 16 x_1 + \frac{3}{2} x_1^2 x_2, \quad \text{and} \quad h_2 = (\beta_{21} - \alpha_{21}) x_1 - (\beta_{11} - \alpha_{11}) x_2 - c_1 = -x_1 - x_2 + 9.
\]

By solving the equations \( h_1(x) = h_2(x) = 0 \), the network has three nondegenerate positive steady states: \( x^{(1)} = (4 - \sqrt{13}, 5 + \sqrt{13}), \ x^{(2)} = (1, 8), \ x^{(3)} = (4 + \sqrt{13}, 5 - \sqrt{13}) \). It is straightforward to check by Lemma 3.2 that \( x^{(1)} \) and \( x^{(3)} \) are stable. Similarly, we can show the networks in Rows (8)–(10) admit multistability. We present the computation in a supporting file, see “witness2.mw” in Table 5.

“\( \Rightarrow \): By Theorem 3.15 and [13, Theorem 3.6 2(b)], if \( G \in \mathcal{G}_1 \) and \( G \) has only 1 species, then \( G \) admits no multistability. By Lemma 5.5, we only need to show the networks listed in Table 4–Rows (1)–(6) do not admit multistability.

For the network in Table 4–Row (1), the polynomial \( g(x_1) \) defined in (3.7) is

\[
g(x_1) = -(\kappa_1 x_1^2 x_2 - \kappa_2 - \lambda \kappa_3 x_1^3) \big|_{x_2=(x_1+c_1)/2} = -(\kappa_1/2 - \lambda \kappa_3)x_1^3 - c_1 \kappa_1/2 x_1^2 + \kappa_2,
\]

where \( \lambda := -\frac{\beta_{12} - \alpha_{12}}{\beta_{11} - \alpha_{11}} = -\frac{\beta_{22} - \alpha_{22}}{\beta_{21} - \alpha_{21}} > 0 \). The number of sign changes of the coefficients is at most 2 since \( g(x_1) \) has at most 3 terms. By Descartes’ rule of signs, \( g(x_1) = 0 \) has at most 2 positive roots. So, this network admits at most 2 positive steady states and by Theorem 3.14, the network does not admit multistability.

For the network in Table 4–Row (2),

\[
g(x_1) = -(\kappa_1 x_1^2 x_2 - \kappa_2 - \lambda \kappa_3 x_1^3) \big|_{x_2=2x_1+c_1} = (\lambda \kappa_3 - 4 \kappa_1)x_1^3 - 4c_1 \kappa_1 x_1^2 c_1^2 - c_1^2 \kappa_1 x_1 + \kappa_2,
\]

and the interval \( I \) is \( (\max\{0, -c_1/2\}, +\infty) \), where \( \lambda := -\frac{\beta_{12} - \alpha_{12}}{\beta_{11} - \alpha_{11}} = -\frac{\beta_{22} - \alpha_{22}}{\beta_{21} - \alpha_{21}} > 0 \). If \(-c_1/2 < 0, \) then \( c_1 > 0, \) and so the number of sign changes of the coefficients is at most 2 since \( g(x_1) \) has at most 3 terms. By Descartes’ rule of signs, \( g(x_1) = 0 \) has at most 2 positive roots. Similarly, if \(-c_1/2 > 0 \) and \( \lambda \kappa_3 - 4 \kappa_1 > 0, \) then by Descartes’ rule of signs, \( g(x_1) = 0 \) has at most 2 positive roots. If \(-c_1/2 > 0 \) and \( \lambda \kappa_3 - 4 \kappa_1 < 0, \) then

\[
g'(\frac{c_1}{2}) = 3(\lambda \kappa_3 - 4 \kappa_1)x_1^2 - 8c_1 \kappa_1 x_1 - 2c_1 \kappa_1 \big|_{x_1=\frac{c_1}{2}} = \frac{3}{4} \lambda \kappa_3 c_1^2 > 0.
\]
So \(g'(x_1) = 0\) has at most 1 root over the interval \(I\), and hence \(g(x_1) = 0\) has at most 2 roots over \(I\). Above all, the network admits at most 2 positive steady states, and so, by Theorem 3.14, the network does not admit multistability. Similarly, we can show that the network in Table 4-Row (3) does not admit multistability.

For the network in Table 4-Row (4), the polynomial \(g(x_1)\) is
\[
g(x_1) = -\left(\kappa_1 x_1^2 - \kappa_2 x_2 - \lambda \kappa_3 x_1^3\right)
= -\left(\kappa_1 - \lambda \kappa_3\right)x_1^3 - 2c_1 \kappa_1 x_1^2 - (\kappa_2 - \kappa_1) x_1 + c_1 \kappa_2,
\]
where \(\lambda := -\frac{\beta_{12} - \alpha_{12}}{\beta_{11} - \alpha_{11}} = -\frac{\beta_{22} - \alpha_{22}}{\beta_{21} - \alpha_{21}} > 0\). Note that for any \(\kappa_1 > 0\), \(\kappa_2 > 0\) and for any \(c_1 \in \mathbb{R}\), \(-2c_1 \kappa_1\) and \(\kappa_2 \kappa_3\) have different signs if \(c_1 \neq 0\). So the number of sign changes of the coefficients of \(g(x_1)\) is at most 2. By Descartes’ rule of signs, \(g(x_1) = 0\) has at most 2 positive roots. So, this network has at most 2 positive steady states and by Theorem 3.14, the network does not admit multistability.

For the network in Table 4-Row (5), the polynomial \(g(x_1)\) is
\[
g(x_1) = \kappa_1 - \kappa_2 x_1 x_2 - \lambda \kappa_3 x_1^3\big|_{x_2=x_1+c_1},
\]
and the interval \(I\) is \((\max(0,c_1),+\infty)\), where \(\lambda := -\frac{\beta_{12} - \alpha_{12}}{\beta_{11} - \alpha_{11}} = -\frac{\beta_{22} - \alpha_{22}}{\beta_{21} - \alpha_{21}} < 0\). So the number of sign changes of the coefficients of \(g(x_1)\) is at most 2. By Descartes’ rule of signs, \(g(x_1) = 0\) has at most 2 positive roots. So, by Theorem 3.14, the network does not admit multistability. Similarly, we can show that the network in Table 4-Row (6) does not admit multistability. □

6. Discussion. For the future work, we propose the problems below.
(1) Does there exist a network \(G\) in \(\mathcal{G}\) such that \(cap_{pos}(G) = 3\) but \(cap_{stab}(G) < 2\)? Remark that for all small networks we have studied (see Table 2 and Table 4), if a network admits three positive steady states, then there are two stable ones.
(2) Under which conditions does a network in $\mathcal{G}$ admit strictly more than 3 positive steady states?

(3) For the set of networks $\mathcal{G}_i$ ($i \in \{0, 1, 2\}$), which subset is the smallest such that any network in this subset admits strictly more than 3 positive steady states?

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SUPPLEMENTARY MATERIAL

Table 5 lists all files at the online repository: https://github.com/HaoXUCode/MSRN-Supplement

| Name            | File Type | Results              |
|-----------------|-----------|----------------------|
| SM.pdf          | PDF       | Descartes’ rule and Theorem 2.7 |
| witness2.mw/.pdf| Maple/PDF | Theorem 2.6           |
| reverse4.mw/.pdf | Maple/PDF | Lemma 5.5            |
| reverse3.mw/.pdf | Maple/PDF | Lemma 5.5            |
| reverse2.mw/.pdf | Maple/PDF | Lemma 5.5            |
| reverse1.mw/.pdf | Maple/PDF | Lemma 5.5            |
| witness1.mw/.pdf | Maple/PDF | Theorem 2.5           |
| irreversible.mw/.pdf | Maple/PDF | Lemma 4.14         |