Spatiotemporal Symmetry in Rings of Coupled Biological Oscillators of *Physarum* Plasmodial Slime Mold

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Spatiotemporal patterns in rings of coupled biological oscillators of the plasmodial slime mold, *Physarum polycephalum,* were investigated by comparing with results analyzed by the symmetric Hopf bifurcation theory based on group theory. In three-, four-, and five-oscillator systems, all types of oscillation modes predicted by the theory were observed including a novel oscillation mode, a half period oscillation, which has not been reported anywhere in practical systems. Our results support the effectiveness of the symmetric Hopf bifurcation theory in practical systems.

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The symmetric Hopf bifurcation theory based on group theory, which was proposed by Golubitsky, Stewart, and Schaeffer [1], provides elegant mathematical understanding of spatiotemporal pattern formation in a coupled oscillator system [1–3]. The analysis by this theory requires only geometrical symmetry, not the intrinsic mechanism of the system; that is, the theory is model independent. As one of its applications, Golubitsky *et al.* investigated animal locomotion generated by central pattern generators. Most of the patterns predicted by this theory correspond to various types of quadrupedal gaits such as a pace, a trot, and a gallop [4]. The theory, however, predicts another novel oscillation pattern, i.e., a half period oscillation, which could not be found in usual animal locomotion. In this study, we investigated a biological coupled oscillator system of a plasmodial slime mold, *Physarum polycephalum,* which consists of three, four, and five oscillators in rings. We show the existence of some of the oscillation patterns predicted by this theory including a half period oscillation. Moreover, we discuss the general effectiveness of the symmetric Hopf bifurcation theory for practical systems by referring to a well known nonlinear chemical oscillator system such as the Belousov-Zhabotinsky (BZ) reaction.

The plasmodium of *Physarum polycephalum* is an amoeboid multinucleated unicellular organism, which shows various oscillatory phenomena, for example, oscillations in adenosine triphosphate and Ca^{2+} concentrations, thickness of the plasmodium, and protoplasmic streaming [5,6]. These oscillation phenomena are supposed to be generated by complicated mechanochemical reactions among the chemicals, actin, and intracellular organelles, etc. However, the detailed mechanism of the generation of the oscillations in the plasmodium has not been elucidated. That is the reason we introduced the group theoretic approach in this study.

From the viewpoint of nonlinear dynamics, the plasmodium can be modeled as a coupled nonlinear oscillator system [7,8], where an oscillator corresponds to each partial body in the plasmodium. In order to construct the coupled oscillator system of the plasmodium, we patterned it with a microfabricated structure (Fig. 1a) by a photolithography method [7,9]. The system consists of several plasmodial oscillators and coupling parts. Figure 1b shows an example of a ring of three oscillators numbered 0, 1, and 2. The oscillators are physically connected by the protoplasmic streaming in the tube structure of the coupling part. With this method, the arrangement of the oscillators and coupling configuration can be systematically controlled by the channel length (L) and width (W). Here the channel width can be regarded as the coupling strength among the coupled oscillators [7].

We investigated thickness oscillation in rings of three, four, and five oscillators with nearest neighbor coupling, where the oscillators and couplings were almost identical and geometrically symmetrical. In all experiments, the channel configurations were fixed at $W = 0.4$ mm and $L = 4$ mm.

In the three-oscillator system, we observed three types of oscillation modes, i.e., a rotation, a partial in-phase, and a half period oscillation. Figures 1c and 2a show the rotation mode, where the rotating wave was observed in the order, oscillator 1, 2, and 0, as shown in Fig. 1c, or sometimes in the opposite direction. The phase differences between neighboring oscillators were all about 120° (the thin line in the plots of phase difference indicates 120°).

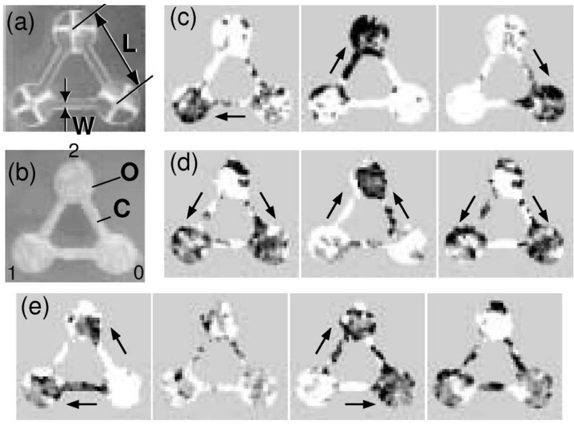

FIG. 1. A ring of three oscillators. (a) Microfabricated structure. (b) Plasmodial coupled oscillator system patterned by the microfabricated structure, which was made from an ultrathick photoresist resin (NANO^{TM} SU-8 25, 50, Microlithography Chemical Corp.) by a photolithography method. The structure consisted of two layers: the first layer 25 μm thick and the second 100 μm thick. The second layer had an opening composed of three circles and channels. The first layer was cross shaped to support the central part surrounded by the three channels. The structure was put on a 1.5% agar plate to pattern the wet (agar) and dry (microfabricated structure) surfaces. The plasmodium spread only on the wet surface to form oscillator parts (**O**) and coupling parts (**C**). The thickness of the first layer was sufficiently small to avoid disturbing the oscillation of the plasmodium in the oscillator part. (c)–(e) Oscillation modes in the thickness of the plasmodium: (c) rotation, (d) partial in-phase, (e) half period mode. The thickness data were obtained from the transmitted light intensity through the plasmodium. Black/white images indicate increase/decrease in thickness. $W = 0.4$ mm; $L = 4$ mm. Time intervals between these images were 27, 56, and 25 sec in (c), (d), and (e), respectively. Arrows indicate the directions of wave propagation in the channels.

Figures 1d and 2b show the partial in-phase mode, where two oscillators (0 and 1) were in phase and the other one (2) was in antiphase against the first two. The most interesting case was the half period mode (Figs. 1e and 2c), where two oscillators (0 and 1) were in antiphase and the third one (2) oscillated twice while the first two oscillated once, namely, the third oscillator had a half period against those of the first two and the antiphase pair and the third oscillator locked at 1:2 frequency ($\omega_{0,1}$:ω_2 in the legend of Fig. 2). In addition, these three oscillation modes spontaneously switched in one successive observation.

In the four-oscillator system, we observed the following four types of oscillation modes. Figure 3a shows the rotation mode. The phase differences between neighboring oscillators were all about 90°. In addition, the half period mode was observed in this system, too, as shown in Fig. 3b, where two diagonal oscillators (0 and 2) were in antiphase and the other two (1 and 3) oscillated in a half period against the first two. Figure 3c shows the partial in-phase/antiphase mode, where two pairs of two adjacent oscillators (0 and 1, 2 and 3) were in phase while the pairs were in antiphase with each other. Figure 3d shows the nearest neighbor antiphase mode, where all pairs of the nearest neighbor oscillators were in antiphase. Although

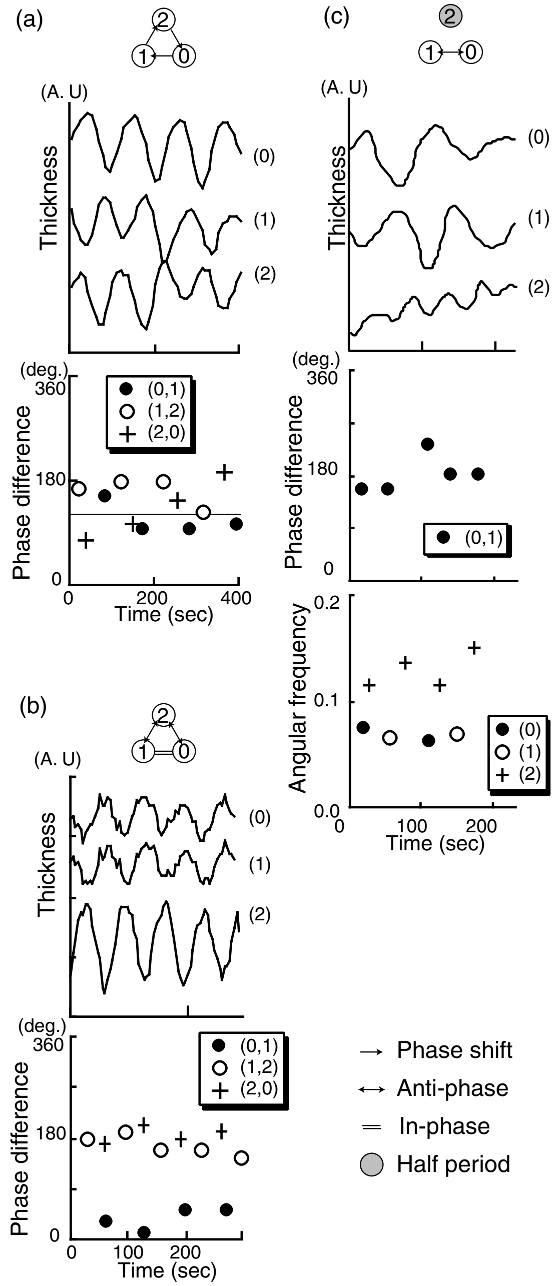

FIG. 2. Oscillation modes in three oscillators: (a) rotation, (b) partial in-phase, (c) half period mode. The diagrams show the oscillation patterns in the three oscillators. A circle with number i denotes oscillator i corresponding to those in Fig. 1b and in the following traces. A gray circle indicates that the oscillation period is half of those in the other oscillators. Unidirectional arrows, bidirectional arrows, and double lines denote phase shift, antiphase, and in-phase oscillations, respectively. The upper traces show time courses of the thickness oscillations obtained by taking averages of the transmitted light images from each oscillator part. The lower plots show the phase differences between any two oscillators. The phase differences were calculated from the time positions of the peaks in the thickness traces. The lowest plots in (c) show the angular frequencies of oscillators. Filled circles, open circles, and crosses denote the frequencies of oscillators 0, 1, and 2, respectively; the time averaged values were $\omega_0 = 0.069 \pm 0.009$, $\omega_1 = 0.067 \pm 0.002$, and $\omega_2 = 0.129 \pm 0.017$, respectively.

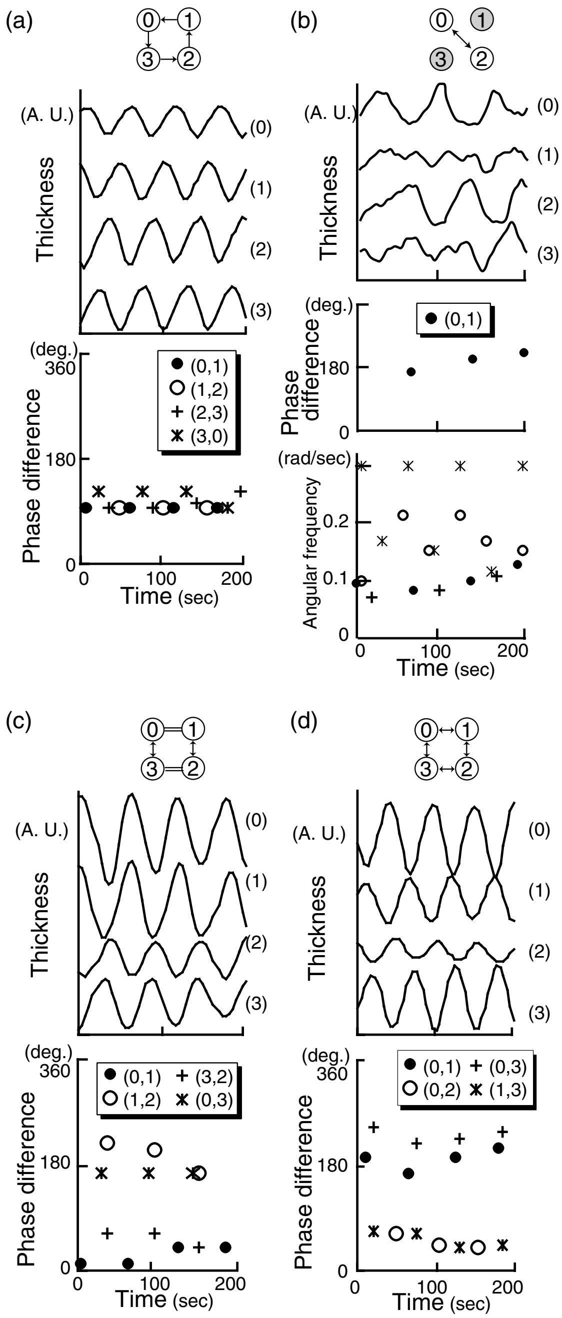

FIG. 3. Oscillation modes in four oscillators: (a) rotation, (b) half period, (c) partial in-phase/antiphase, (d) nearest neighbor antiphase mode. Notations are the same as in Fig. 2 except for the angular frequencies. Filled circles, open circles, crosses, and asterisks denote the frequencies of oscillators 0, 1, 2, and 3, respectively; the time averaged values were $\omega_0 = 0.099 \pm 0.016$, $\omega_1 = 0.185 \pm 0.051$, $\omega_2 = 0.086 \pm 0.016$, and $\omega_3 = 0.208 \pm 0.090$, respectively.

we do not mention it here, we observed richer patterns, which will soon be reported elsewhere with further group theoretic analysis.

In the five-oscillator system, we observed the following three types of oscillation modes. Figures 4a and 4b show the rotation modes. The phase differences between neighboring oscillators were all about 72° in Fig. 4a but about

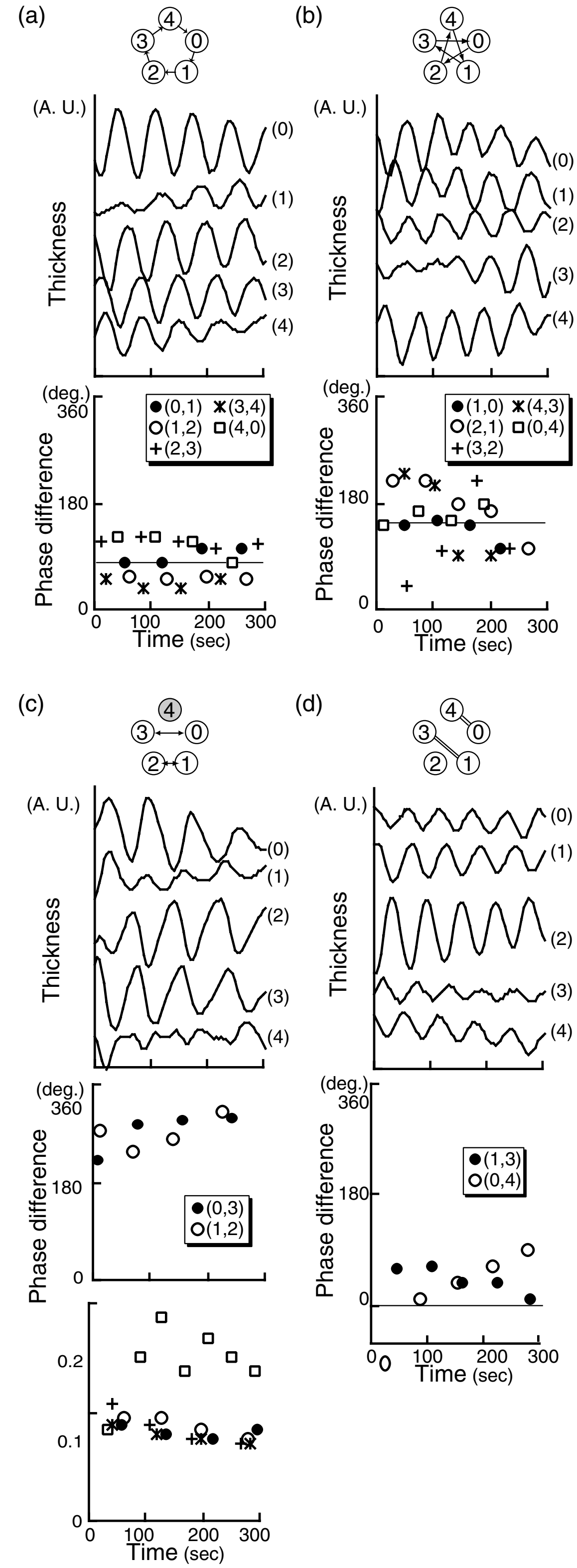

FIG. 4. Oscillation modes in five oscillators: (a) rotation with phase shift 72°, (b) rotation with phase shift 144°, (c) half period, (d) partial in-phase mode. Notations are the same as in Fig. 2 except for the angular frequencies. Filled circles, open circles, crosses, asterisks, and squares denote the frequencies of oscillators 0, 1, 2, 3, and 4, respectively; the time averaged values were $\omega_0 = 0.082 \pm 0.006$, $\omega_1 = 0.090 \pm 0.006$, $\omega_2 = 0.090 \pm 0.016$, $\omega_3 = 0.079 \pm 0.009$, and $\omega_4 = 0.155 \pm 0.020$, respectively.

144° in Fig. 4b. In the latter case, the wave propagated drawing a trace like a star in the order: oscillators 0, 2, 4, 1, and 3. The half period mode was observed even in this system, as shown in Fig. 4c, where two facing pairs of two oscillators (0 and 3, 1 and 2) were in antiphase (although not perfectly) and the fifth one (4) oscillated in a half period against the first four oscillators. Figure 3d shows the partial in-phase mode, where two pairs of two facing oscillators (0 and 4, 1 and 3) were in phase. The mode switching was observed in both the four- and five-oscillator system.

These plasmodial oscillator systems can be regarded as a D_n symmetric ring of n identical nonlinear oscillators with identical two-way nearest neighbor couplings. The analysis with the symmetry-breaking Hopf bifurcation theory shows that there are several branches, each corresponding to a different isotropy subgroup of $D_n \times S^1$ [1–3]. All the oscillation modes we observed in this study were predicted by this analysis and each mode corresponds to one demanded by an isotropy subgroup in the theory (compare our results with the lists reported in Table I of Ref. [2] for a three-oscillator system, and in Figs. 8.1 and 8.2 of Ref. [3] for four- and five-oscillator systems, respectively). Moreover we observed spontaneous switching between those oscillation modes, which suggests that the modes are multistable. In the plasmodial system, the coupling strength or the state of the oscillators seems to fluctuate, which could affect the switching.

The rotation mode has already been observed in the BZ reaction system, such as a three-coupled continuous-flow stirred tank reactor (CSTR) system [10] and spatially distributed three-cation-exchange beads system [11]. Only the rotation mode was given as a stable solution by the former analysis [12]. However, richer oscillation modes were reported in the three-coupled CSTR system by Yoshimoto *et al.* [10]. One is the death in-phase mode, another is the death antiphase one, where two oscillators are in-phase/antiphase and the third shows the mode they called "death," which means amplitude death. Although they regarded the oscillation in the third oscillator as "death" in Ref. [10], the oscillators still have a small amplitude. In the death in-phase mode, the third oscillator had the same period as the other two (Fig. 6c in Ref. [10]). It is noteworthy that, in the death antiphase mode, the third oscillator sometimes showed the half period oscillation (Fig. 6d in Ref. [10]). Therefore, it would be natural to classify these two oscillation modes into the partial in-phase and half period modes mentioned above. The symmetric Hopf bifurcation theory also guarantees these two oscillation modes; namely, the theory provides a list of possible solutions including ones that could not be found by the conventional analysis.

In conclusion, we elucidated that all the oscillation modes predicted by the symmetric Hopf bifurcation theory were commonly observed in practical systems including both the chemical reaction system and the biological system. This approach will be widely effective for analyzing the dynamics of geometrically symmetric systems encountered in various practical systems. It should be emphasized that this approach clarifies the possible oscillation modes only by the symmetry group of the system and needs no specific information about individual elements. The theory will provide a perspective of the dynamics of systems that will avoid overlooking possible modes and will help to capture the intrinsic mechanism.

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