Cyclic dominance emerges from the evolution of two cooperative behaviours in the social amoeba

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Abstract

Evolution of cooperation has been one of the most important problems in sociobiology, and many researchers have revealed mechanisms that can facilitate the evolution of cooperation. However, most studies deal only with one cooperative behaviour, even though some organisms perform two or more cooperative behaviours. The social amoeba *Dictyostelium discoideum* performs two cooperative behaviours in starvation: fruiting body formation and macrocyst formation. Here, we constructed a model that couples these two behaviours, and we found that the two behaviours are maintained due to the emergence of cyclic dominance, although cooperation cannot evolve if only either two behaviours is performed. The common chemoattractant cyclic AMP is used in both fruiting body formation and macrocyst formation, providing a biological context for this coupling. Cyclic dominance emerges regardless of the existence of mating types or spatial structure in the model. In addition, cooperation can re-emerge in the population even after it goes extinct. These results indicate that the two cooperative behaviours of the social amoeba are maintained due to the common chemical signal that underlies both fruiting body formation and macrocyst formation. We demonstrate the importance of coupling multiple games when the underlying behaviours are associated with one another.

Introduction

Evolution of cooperation has been a challenging topic in sociobiology. Although it is a widespread behaviour, apparent from human societies [7] to microbial communities [49], cooperation is vulnerable to invasion by defectors. Cooperators pay a cost for helping other individuals, while defectors pay no cost but receive the benefits from cooperators. As a consequence, natural selection should favour defectors [26, 50]. However, many studies have revealed mechanisms that facilitate the evolution of cooperation, including kin discrimination [19, 21, 36], spatial structure that enables cooperators to interact frequently with one another [2, 8, 24, 25], and a by-product which increases the benefit for cooperators (e.g., pleiotropy [10, 23, 45, 46]).

Most studies have explored only one cooperative behaviour at a time. However, some organisms, such as the social amoeba *Dictyostelium discoideum*, perform multiple types of cooperation. It has been known that *D. discoideum* performs two distinct types of cooperative behaviours in starvation. One of these is fruiting body formation, wherein some cells differentiate into spore cells and survive while other cells differentiate into nonviable stalk cells and aid in dispersal of spore cells [35]. The offspring germinate from spore cells after conditions become favorable [20]. The other cooperative behaviour is known as macrocyst formation, wherein some cells become sexually mature and produce offspring after forming diploid zygote cells, but other cells remain vegetative and provide energy to the zygote cells through cannibalism [28]. The haploid progeny eventually appears, but the germination process remains unclear [28].

In fruiting body formation, cells first aggregate by responding to synchronously secreted cyclic AMP (cAMP) [13], and then, the aggregated cells form multicellular slugs and develop into fruiting

bodies. When cells from two or more strains form a fruiting body (i.e., forming a chimeric fruiting body), the cell-type ratio has a large effect of the fitness of each strain [37, 31]. If cells from one strain differentiate only into spore cells, this strain can receive a large benefit through the formation of chimeric fruiting bodies because they pay no cost for stalk formation. As differentiation is key to fruiting body formation, theoretical studies have focused on signaling chemicals that induce stalk formation [29, 44].

In macrocyst formation, about 16% of cells first mature sexually and differentiate into gamete or fusion component (FC) cells [27], whereas other cells remain vegetative. In the heterothallic strains of *D. discoideum*, there are three mating types, and each FC cell fuses with that of a different mating type [3]. After cell fusion, pronuclear fusion occurs [17] and zygote cells are formed. The zygote cells then secrete cAMP [1] to collect the vegetative cells around them for cannibalization, which leads to mature zygote cells in the macrocysts. As the zygote cells produce offspring while the vegetative cells are being cannibalised by the zygote cells, macrocyst formation is considered as a form of cooperation. In contrast to fruiting body formation, however, there are fewer studies on the evolution of macrocyst formation, likely due to difficulties in germinating macrocysts in the laboratory [11]. To our knowledge, there are only two theoretical studies on macrocyst formation; one shows that fluctuations in food availability play an important role in maintaining the ability to aggregate the vegetative cells [6], and the other indicates that without food fluctuation, vegetative cells would still respond to cAMP if macrocyst formation co-occurs with fruiting body formation and if cooperation in fruiting body formation is preserved [33].

Here, we use a modeling approach to demonstrate that the two cooperative behaviours of *D. discoideum*, fruiting body formation and macrocyst formation, can be maintained by coupling the evolution of these two behaviours. First, we considered a very simple situation that ignored mating types and spatial structure. In this model, cyclic dominance, a loop structure where one strategy beats another strategy but this strategy is beaten by still another strategy, emerged under some conditions (see inequality (7) and figure 2), and cooperation in both fruiting body formation and macrocyst formation was maintained. We then introduced variation in mating types and assumed that macrocyst formation occurred only between mating types. Cyclic dominance again emerged in this case. When the model included spatial structure, such that each colony of the social amoeba interacted only with their neighbours, this result held and cooperation was maintained. Thus, by coupling these cooperative behaviours, cyclic dominance emerges and both behaviours are maintained even if it is assumed that neither cooperative behaviour can evolve alone. These results are consistent regardless of mating types and spatial structure introduced to the model if the environment where fruiting body formation co-occurs with macrocysts is assumed.

Models

In this section, we describe the methods for the simulations we performed. The materials and methods for the experiments conducted in this study can be found in the Supplementary information (SI).

Model assumptions

As both fruiting bodies and macrocysts are formed under dark and dry conditions [33] (see also our experimental results in SI and figure S7), we assume that *D. discoideum* can perform both fruiting body formation and macrocyst formation. Note that we ignore homologous recombination during macrocyst formation. For simplicity, both fruiting body formation and macrocyst formation are represented as a prisoner's dilemma (PD) game, whose payoff matrices are given by

	Cooperate	Defect
Cooperate	a_i	0
Defect	b_i	ϵ_i

(1)

where $i = 1$ corresponds to fruiting body formation, and $i = 2$ refers to macrocyst formation. It should be noted that the inequality $b_i > a_i \gg \epsilon_i > 0$ is satisfied for $i = 1, 2$ and that cooperation cannot evolve if only either of the two games is performed.

Cooperators of fruiting body formation are those that produce both spore cells and stalk cells, whereas defectors produce only spore cells. When cooperators form fruiting bodies with another cooperator, they receive benefit a_1. On the other hand, defectors receive b_1 when they exploit the cooperators by forming chimeric fruiting bodies, but the cooperator receives no benefit in this case. However, when defectors interact with another defector, they receive only a small benefit ϵ_1 because they both are deficient in stalk formation. Such a mutant strain is referred to as *fbxA*$^-$ in *D. discoideum* [12].

In macrocyst formation, cooperators provide energy to zygote cells by making vegetative cells respond to cAMP to allow cannibalization by zygote cells. On the other hand, defectors avoid cannibalistic attack by ignoring cAMP. Such a mutant strain is known as TMC1 [34]. When the two cooperators produce macrocysts together, they each receive a benefit a_2, as macrocysts obtain enough energy through cannibalism. On the other hand, if a defector interacts with a cooperator, the defector receives b_2 by avoiding a cannibalistic attack, but the cooperator receives no benefit. However, when the two defectors form macrocysts together, they receive a very small benefit ϵ_2, as macrocysts cannot receive enough energy from the vegetative cells alone.

It should be noted that the common chemical signal cAMP plays an important role in both fruiting body formation and macrocyst formation; aggregation requires cAMP in both cases. If cells do not aggregate in macrocyst formation (i.e., a defector of macrocyst formation), they do not aggregate in fruiting body formation either. In other words, defectors during macrocyst formation cannot join in fruiting body formation. This phenomenon was experimentally identified [34], and we reflect it in the models (ϵ_0 in payoff matrix given by equaiton (2)).

We began by building a very simple model, where mating types and spatial structure were ignored. In this case, there were only three strategies: a cooperator of both fruiting body formation and macrocyst formation (C), a defector of fruiting body formation that behaved as a cooperator of macrocyst formation (D^F), and a defector of macrocyst formation that could not join in fruiting body formation (D^M). The payoff matrix A is given by

$$\begin{array}{c|ccc} & C & D^F & D^M \\ \hline C & (1-\theta)a_1 + \theta a_2 & \theta a_2 & (1-\theta)ha_1 \\ D^F & (1-\theta)b_1 + \theta a_2 & (1-\theta)\epsilon_1 + \theta a_2 & (1-\theta)h\epsilon_1 \\ D^M & (1-\theta)\epsilon_0 + \theta b_2 & (1-\theta)\epsilon_0 + \theta b_2 & (1-\theta)\epsilon_0 + \theta\epsilon_2 \end{array} \tag{2}$$

where θ is the probability that macrocyst formation occurs, while $1 - \theta$ refers to fruiting body formation ($0 < \theta < 1$). ϵ_0 is the payoff for remaining as solitary vegetative cells in fruiting body formation. For simplicity, we assume that starvation continues for a long period, where almost all vegetative cells die [9]. In this case, it is reasonable to assume that $\epsilon_0 \ll \min\{\epsilon_1, \epsilon_2\}$. h denotes the payoff effect for forming small fruiting bodies ($0 < h \leq 1$). When a cooperator of both fruiting body formation (C) (or a defector of fruiting body formation (D^F)) forms fruiting bodies with a defector of macrocyst formation (D^M), D^M cannot join in fruiting body formation because it cannot respond to cAMP. The number of cells within fruiting bodies is thus smaller than that when C or D^F interacts with either of them. Such fruiting bodies would be shorter and provide lower payoffs due to the inefficiency of dispersal. The roles for additional parameters θ, ϵ_0, and h are shown in figure 1.

Next, we introduced mating types (type 1 and type 2). In this model, there exist six strategies (C_1, D_1^F,D_1^M, C_2, D_2^F, and D_2^M), where the subscripts indicate the mating types of each strategy (e.g., C_1 represents a cooperator with mating type 1). Within the matin types, only fruiting body formation is performed, wherease both fruiting body formation and macrocyst formation are performed between the mating types. Note that θ represents the probability that macrocyst formation is performed between the mating types. In the model including mating types., therefore,

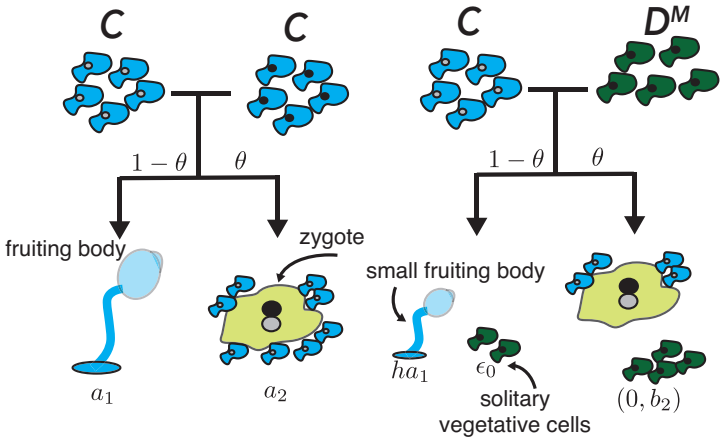

Figure 1: Summary of the additional parameters θ, ϵ_0, and h. When the two strategies interact, they form macrocyst formation with a probability of θ or fruiting body formation with a probability of $1-\theta$. If C forms chimeric fruiting bodies with D^M, C receives the payoff ha_1 because fruiting bodies are shorter than when two Cs form fruiting bodies (compare the left case), while D^M receives ϵ_0, as it cannot join in fruiting body formation. Note that D^M can exploit C for macrocyst formation, and D^M receives b_2, while C receives no benefits in this case.

the payoff matrix S is described as

$$S = \begin{pmatrix} A(0) & A(\theta) \\ A(\theta) & A(0) \end{pmatrix}, \tag{3}$$

where $A(0)$ is a 3×3 payoff matrix for the same mating type strategies (they perform only fruiting body formation), while $A(\theta)$ refers to the payoff matrix for different mating type players that perform macrocyst formation with the probability of θ.

Replicator Dynamics

The continuous and discrete replicator dynamics are defined, respectively:

$$\frac{dx_i}{dt} = x_i \left\{ \left(P\mathbf{x}^{\mathrm{T}}\right)_i - \mathbf{x}P\mathbf{x}^{\mathrm{T}} \right\} \tag{4}$$

$$x_i(t+1) = \frac{\left(P\mathbf{x}^{\mathrm{T}}\right)_i}{\mathbf{x}P\mathbf{x}^{\mathrm{T}}}. \tag{5}$$

where x_i is the frequency of strategy i, $\mathbf{x}$ is a vector of the frequencies of each strategy ($\mathbf{x} = (x_i)$), and P is a payoff matrix. When the mating types are ignored, P equals to the payoff matrix A given by equation (2) while P equals to the payoff matrix S by equation (3) if mating types are included. Numerical computation for continuous replicator dynamics was conducted in Python using the integrators of "ode" with "dopri5" or "odeint" in the Scipy package [18].

Agent-Based Model

One of the advantages of using an agent-based model is to introduce spatial structure. As macrocyst formation occurs only between mating types, spatial structure can have an effect on the frequencies of fruiting body formation and macrocyst formation. For example, only fruiting body formation would occur if all colonies in the neighbourhood have the same mating type.

In the agent-based model, each agent represents a small colony of *D. discoideum* that has one of the six strategies (C_1, D_1^F,D_1^M, C_2, D_2^F, and D_2^M) because mating types are also included in

this model. By introducing the torus lattice model and Moore neighbourhood, each agent interacts with their eight neighbours at one time step. After all agents finish their interaction and their total payoffs are calculated, they synchronously update their strategies to those of their neighbours with some probabilities given below. In many studies, the Fermi function is used as a stochastic update process [38, 47], but the Fermi function is for the pairwise update process. Therefore, we defined the probability Π_{ij} that agent i adopts the strategy of agent j using the softmax function

$$\Pi_{ij} = \frac{\exp\{(p_j - p_i)/K\}}{\sum_{k \in N_i} \exp\{(p_k - p_i)/K\}}, \tag{6}$$

where p_i is the total payoff of agent i, N_i is the set of agents in the neighbourhood of agent i (including agent i itself), and K is the inverse of the intensity of selection. It is more likely that agent i adopts the strategy of agent j in the next time step if $p_i < p_j$, but agent i stochastically may adopt the strategy of agent l even if $p_i > p_l$. At the $K \to 0$ limit, agent i adopts only the strategy of the agent whose payoff is the highest in the neighbourhood. By contrast, as K increases, it is more likely that each agent randomly adopts a strategy in their neighbourhood (selection does not work in the $K \to \infty$ limit).

We performed the pairwise invasion analysis at 100×100 lattice points. One of the two strategies is called an invader, whose initial frequency is 0.01, and the other is a resident. We ran the simulation for $1,000$ time steps at various parameter values, and we replicated 50 times in each case. We then investigated whether the invaders excluded the residents (the invader frequency was greater than 0.99 at the end of the simulation) or they were maintained (the frequencies were greater than 0.01). If invaders excluded the residents, this indicated that the invaders beat the residents. If the invaders did not exclude the residents but they were maintained, they coexisted with the residents.

We then simulated the situations where all six strategies existed at the initial conditions. In this case, we used 300×300 lattice points to reduce stochastic effects. In each simulation, the initial frequencies of all strategies were equal, and simulations were continued for $100,000$ time steps. We replicated the simulation 10 times at each value of θ.

We also investigated the effect of mutation in the agent-based model. We again used 300×300 lattice points, but here we assumed that mutation was rare; at each 500 time step, one agent was randomly chosen to update the strategy to one of the other five strategies, which was again randomly chosen. All codes for the agent-based model were written in C language.

Data Availability

The simulation codes, the Python script for the analysis, and the experimental data are available at GitHub (`https://github.com/ShotaSHIBASAKI/Cyclic-dominance-emerges`).

Results

Excluding mating types and spatial structure

First of all, we built the simple model that does not include mating types nor spatial structure. In this case, payoff matrix given by equation (2) shows a rock-scissors-paper (RSP) game, approximately if

$$\frac{b_2 - a_2}{a_1} < \frac{1-\theta}{\theta} < h^{-1}, \tag{7}$$

see SI for derivation. In other words, when inequality (7) holds, cyclic dominance emerges, where D^F beats C, D^M beats D^F, and C beats D^M. This result can be explained intuitively as follows: in fruiting body formation, C is exploited by D^F because fruiting body formation is assumed to be represented as the PD game (equation (1)). By contrast, in macrocyst formation, D^F is exploited by D^M because we assume that macrocyst formation can be represented by the PD game (equation (1)). It is true that C is also exploited by D^M in macrocyst formation, but C can enjoy fruiting

body formation without exploitation by D^M, which cannot aggregate in fruiting body formation. Therefore, C can beat D^M if the payoff of forming fruiting body and the probability of fruiting body formation are sufficiently large. However, if the probability of fruiting body formation is too large, D^M cannot beat D^F, as fruiting body formation frequently occurs, and D^M does not obtain a large benefit.

In addition, we found that the continuous replicator dynamics [42] has a unique internal equilibrium G, to which any other interior point convergence if inequality (7) holds (figure 2, see SI for detail). In this case, a cooperator of both fruiting body formation and macrocyst formation C is maintained, although it is not an evolutionarily stable strategy [22]. Therefore, cooperation is maintained if there exist two games, even though cooperation cannot evolve when the social amoeba plays ether of the two games.

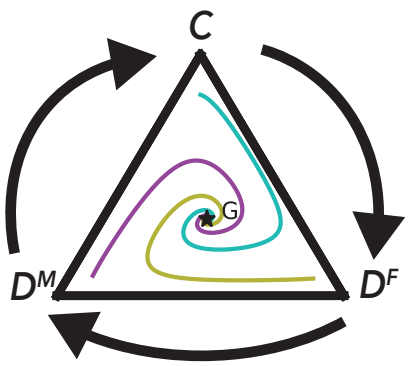

Figure 2: The evolutionary dynamics given by continuous replicator dynamics with payoff matrix [2] when [7] holds. The three orbits (cyan, yellow, and magenta) begin at different initial points but converge at the internal equilibrium G. The parameters are $a_1 = 1$, $b_1 = 1.5$, $\epsilon_1 = 10^{-4}$, $a_2 = 0.5$, $b_2 = 1.2$, $\epsilon_2 = 10^{-4}$, $\theta = 0.5$, $h = 0.5$, and $\epsilon_0 = 10^{-6}$.

It should be noted, however, that the application of continuous replicator dynamics is unlikely to be valid for *D. discoideum* because fruiting body formation and macrocyst formation occur in discrete generations; fruiting body formation and/or macrocyst formation occur through starvation, with the offspring subsequently emerging. Thus, discrete replicator dynamics should be applied. Mathematically, the continuous replicator dynamics is a limited case of discrete replicator dynamics [5], which means the result shown in figure 2 is not always valid when the discrete replicator dynamics is applied. Indeed, we found that with discrete replicator dynamics, the range wherein any orbit converges to the internal point G is narrower than that in the case of continuous replicator dynamics (inequality (7,) and SIfigure S1).

A model that includes mating types

When the model includes the existence of mating types, cyclic dominance emerges again (figure 3). In this case, fruiting body formation occurs both within and between the mating types, while macrocyst formation occurs only between the mating types. This means that the parameter θ is the probability that macrocyst formation occurs between mating types. As shown in figure 3, D^Fs beat both Cs and D^Ms within the mating types because only fruiting body formation occurs and is represented by the PD game (equation (1)). However, D_i^F is beaten by the other mating type D_j^M ($i \neq j$) because D^Fs are exploited during macrocyst formation and receive only a small payoff for fruiting body formation due to the inability to produce stalk cells. By contrast, D^Ms are beaten by the same mating type Cs because D^Ms cannot undergo fruiting body formation due to the lack of the responsibility to cAMP. In addition, under some parameter values, Cs can beat the other mating type D^Fs and D^Ms (see SI).

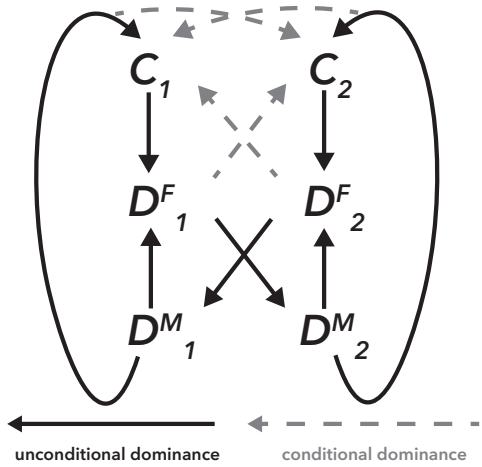

Figure 3: Cyclic dominance emerges when different mating types are introduced. Black arrows show unconditional dominance, and gray arrows show conditional dominance.

Although continuous or discrete replicator dynamics can be applied when mating types are introduced, it is difficult to calculate the stability of an interior equilibrium due to the high dimensionality of the model. In addition, the initial mating type ratio has a large effect on the evolutionary dynamics (figure S2). For these reasons, we used an agent-based model for additional analyses.

A model that includes spatial structure

In the agent-based model, we again found cyclic dominance that was similar to figure 3, and this result is confirmed by the results of the pairwise invasion analysis shown in figure S3–S5. We then analyzed cases wherein all six strategies coexisted equally in the initial conditions. Due to the emergence of cyclic dominance, the cooperators (both C_1 and C_2) were maintained if θ was sufficiently large (figure 4a). When $\theta = 0$, only fruiting body formation occurred within and between mating types. As a consequence, defectors of fruiting body formation (D_1^F and D_2^F) beat the other strategies. If θ was small but non-zero ($0.1 \leq \theta \leq 0.3$), only the defectors of macrocyst formation (D_1^M and D_2^M) were maintained. This is because the probability of macrocyst formation between mating types θ is not sufficiently large for cooperators C_i to obtain the benefits from forming macrocysts with the other mating type cooperators C_j or defectors of fruiting body formation D_j^F ($j \neq i$) (Fig S.3), and because defectors of macrocyst formation can exploit other mating type defectors of fruiting body formation (Fig S5). If θ is sufficiently large ($0.4 \leq \theta \leq 1$), however, the cooperators can beat the other mating type defectors of fruiting body formation (figure S3) and all six strategies can be maintained (figure 4b).

In addition, mutation was introduced to the agent-based model. Under the assumption that mutation occurs in each 500 time steps, only a few strategies can coexist. Here, cooperators can re-invade the population even after they are excluded (figure 5). In the initial condition, two mating types of cooperators (C_1 and C_2) coexist, but C_2 is excluded due to the arrival of the mutant in fruiting body formation (D_2^F). For a short period, only C_1 exists in the population, but the same mating type defector of fruiting body formation D_1^F excludes C_1. After that, the other mating type defector of fruiting body formation D_2^F appears and coexists with D_1^F, which is an evolutionarily stable state if the game for macrocyst formation is ignored [32]. However, this coexistence collapses with the invasion of the defector of macrocyst formation D_2^M (see also figure S6). After that, the cooperator C_1 appears again by mutation and becomes fixed in the population. Thus, cooperators of both fruiting body formation and macrocyst formation can be revived even after they disappear.

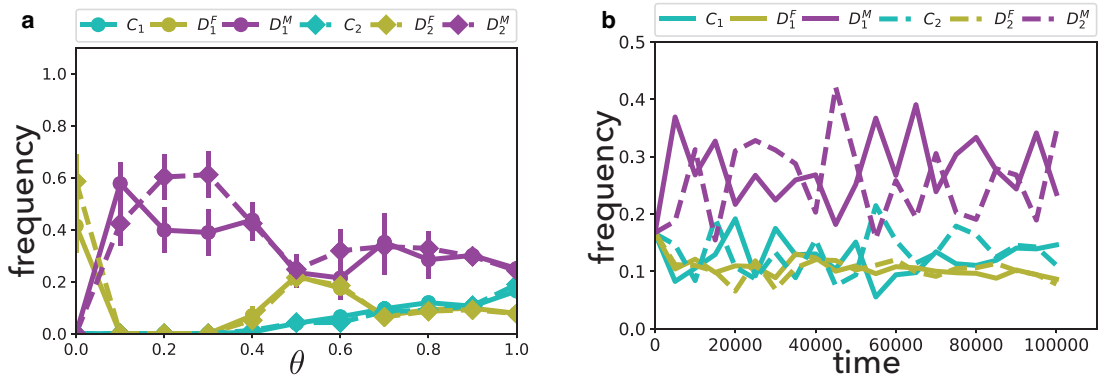

Figure 4: Evolutionary dynamics in the agent-based model. The mean frequencies of each strategy at the end of the simulations with different values of θ are shown in (a). The error bars denote standard errors, but some are too small to see. An example of the evolutionary dynamics when $\theta = 0.9$ is shown in (b). The parameters are $a_1 = 1$, $b_1 = 2.7$, $\epsilon_1 = 10^{-5}$, $a_2 = 0.5$, $b_2 = 1.2$, $\epsilon_2 = 10^{-5}$, $h = 0.5$, $\epsilon_0 = 10^{-6}$, and $K = 1$.

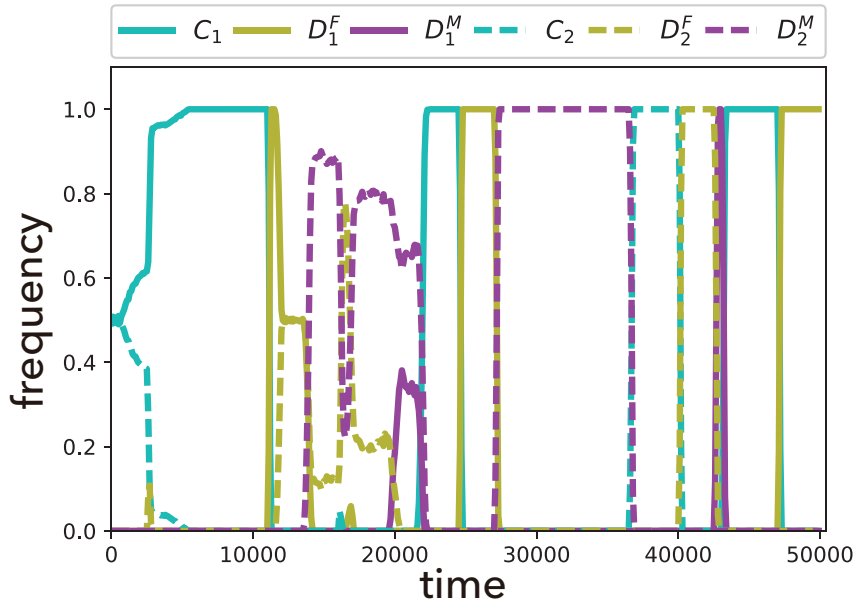

Figure 5: An example of evolutionary dynamics in the agent-based model with mutation. Each color shows the different strategies (cyan C, yellow D^F, and magenta D^M), whereas solid and dashed lines represent the mating types 1 and 2, respectively. It is assumed that mutation occurs rarely: only one agent changes its strategy for each 500 time step. In this situation, only a few strategies coexist, and the cooperators (C_1 and C_2) can invade the population even if they were previously excluded. The parameters are $a_1 = 1$, $b_1 = 2.7$, $\epsilon_1 = 10^{-5}$, $a_2 = 0.6$, $b_2 = 1.2$, $\epsilon_2 = 10^{-5}$, $h = 0.5$, $\epsilon_0 = 10^{-6}$, $\theta = 0.8$ and $K = 0.1$.

Discussion

In this paper, our models demonstrate that the two cooperative behaviours of *D. discoideum*, fruiting body formation and macrocyst formation, can be maintained by coupling the two games. While previous research assumed the maintenance of cooperation in fruiting body formation [33], we show that defectors can appear both in fruiting body formation and macrocyst formation. Even though we assume that both games are represented as PD games (equation 1), which means neither of the two cooperative behaviours can evolve alone, the two cooperative behaviours can be maintained if the model includes both of the games simultaneously.

The primary biological explanation for this result is that both games are not independent; the common chemical signal cAMP is necessary for aggregation in both fruiting body formation (reviewed in [43]) and macrocyst formation [1]. If some cells do not aggregate (i.e., defect) in macrocyst formation, they receive benefits by avoiding the cannibalistic attack by zygote cells. On the other hand, these cells have substantial costs because they cannot participate in fruiting body formation. Indeed, a mutant that weakly expresses a cAMP receptor will result in poor aggregation for both fruiting body formation and macrocyst formation [34]. This phenomenon is represented as ϵ_0 in the payoff matrix (equation 2), and this provides an explanation why the cooperator of both fruiting body formation and macrocyst formation (C) can beat the defector of macrocyst formation (D^M) in the most simple model, wherein the existence of mating types and spatial structure is ignored (figure 2).

The key factor for the maintenance of the cooperators is the emergence of cyclic dominance. Under a simple model without the inclusion of mating types and spatial structure, if the inequality (7)is satisfied, the payoff matrix given by equation (2) follows an RSP game, which is a famous example of cyclic dominance [39]. In addition, the continuous replicator dynamics show the existence of the global attractor in this model (figure 2), and the discrete replicator dynamics shows a similar result (figure S1). Moreover, even when mating types are introduced, cyclic dominance continues to emerge (figure 3). Within mating types, the defectors of fruiting body formation (D^Fs) beat the other two strategies because they perform only fruiting body formation. However, D^Fs lose to other mating types of the defectors of macrocyst formation (D^Ms). Furthermore, D^Ms are beaten by the same mating type cooperators C, because Cs can undergo fruiting body formation without exploitation by D^Ms. The results of the model with spatial structure are also consistent. Cyclic dominance appears again, although almost all instances are dependent on the parameter values (i.e., conditional advantage, figure S3–S5). Due to cyclic dominance, all six strategies can coexist (figure 4), and cooperators can re-emerge even after they are excluded (figure 5).

The approach we employed in this paper is referred to as multi-games [15, 47, 40] or mixed games [48], wherein players play two or more games with different payoff matrices. To our knowledge, there exists no study that applies multi-games to specific biological examples, but our model shows that these approaches provide a meaningful examination for two cooperative behaviours in the social amoeba *D. discoideum*; these behaviours can be maintained by coupling the two games.

It should be noted, however, that our results are based on the assumption that fruiting bodies and macrocysts coexist. Although this has been shown under dark and relatively dry conditions [33], macrocysts are not formed under light conditions [14] and fruiting body formation is inhibited in water [4]. Under these conditions, *D. discoideum* undergoes only one or the other behaviour, and cooperative behaviours are not maintained. In other words, the probability of macrocyst formation θ changes over time under changing environments, and cooperation between fruiting body formation and macrocyst formation would be destabilized. Indeed, our model shows that cooperation of fruiting body formation and macrocyst formation is not always maintained under dynamic environments (SI and figure S8). Future research should consider how changing environmental conditions would affect these conclusions.

In addition, our models are a simplification of the complex behaviours exhibited by *D. discoideum*. For example, we assumed distinct strategies (to cooperate or to defect) for both fruiting body formation and macrocyst formation, but these strategies are continuous. Productivity and sensitivity to signals for differentiation in fruiting body formation [29, 44], or the ratio of FC cells

and vegetative cells in macrocyst formation [33] can be considered. In addition, we assumed that starvation continues for an extended period of time. If this assumption was relaxed, our results might change because a lack of aggregating cells can be beneficial for fruiting body formation and macrocyst formation if the resource condition quickly recovers [41]. Under this scenario, it may be possible that defectors of macrocyst formation (D^Ms) can beat cooperators (Cs), which would lead to the collapse of cyclic dominance.

Moreover, our results do not contradict with those of previous studies. For example, kin discrimination by the cell adhesion proteins TgrB1 and TgrC1 [16] and the greenbeard effect encoded by *csA* [30] are important for maintenance of cooperation in fruiting body formation. If these effects are included in a model, cooperation would be maintained. Our results, on the other hand, suggest that cooperation can be maintained even if such effects do not exist or if they are incomplete. In addition, if kin discrimination and/or the greenbeard effect are introduced in our models and D^Fs do not spread, defectors of macrocyst formation (D^Ms) may be unable to invade and only cooperators (Cs) will be maintained because D^Ms cannot beat Cs.

This study suggests the idea of coupling multiple types of cooperation. This point may be important beyond the social amoeba. For example, there exist a variety kinds of helping behaviours in humans [7]. If some of these behaviours can be regarded as different types of cooperation and if some relations underlie among them, coupling of them may provide a new insight to the evolution of cooperation in humans.

Conclusion

In summary, we propose that cooperative behaviours in the social amoeba can be maintained by coupling the two games. This is because cyclic dominance emerges from the common cAMP signal that is necessary for aggregation in both behaviours. This result is consistent when mating types and spatial structure are introduced into the model, suggesting the generality of these findings and the importance of including multi-games in behavioural models, especially if the games are not independent.

Competing interests

Authors declare no conflict of interest.

Authors' contributions

S.S. and M.S. designed the research, S.S. performed the research and analyzed the data, and S.S. and M.S. wrote the paper.

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