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Endosymbiosis as a compact ecosystem with material cycling: Parasitism or mutualism?

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Abstract

To discover the evolutionary logic of intracellular endosymbiosis, we investigated a theoretical model (simultaneous ordinary differential equations) of a material-cycling system inside a host cell. In the model, we introduced a recently developed cell biology concept called ''autophagy'', which is a decomposing–recycle process of self-compiled materials found universally among eukaryote cells. Our model is based on traditional simultaneous ODE for natural ecosystems that involve producing, grazing, and decomposing processes in material cycling. In the basic intracellular metabolic system, several enzymes regulate metabolism by synthesizing and converting metabolites into biomolecules that are precursors for enzymes involved in the producing process. Symbionts are involved in grazing processes and autophagosomes that degrade materials are involved in decomposing recycles. We compared and analyzed the local stability of ODE systems in three cases: (1) the independent, free-living cell (the basal state of a cell), (2) the case where symbionts invade and exploit macrobiomaterials as parasites inside a host cell, and (3) the combination where symbionts assist the host's metabolism. We conclude that: (i) as consumers, symbionts are required to have a growth rate that is higher than the rate of autophagosome decomposition, (ii) the host cell with a biomass larger than the threshold size would realize the mutualistic relationship with its symbiont, and (iii) this partnership accelerates the biomaterial turnover flow on the basis of biomaterials.

Keywords: Turnover flow; Autophagy; Decomposing process; Metabolic system; Evolution

1. Introduction

Endosymbiosis, a biotic interaction wherein a symbiont inhabits inside its host cell or digestive organs, is universally recognized in nature (Buchner, 1965). In the evolution of endosymbiosis, a new mutualistic system emerges from phylogenetically distinct organisms, often generating wide adaptive radiation. Endosymbiosis is considered one of the major transitions in evolution (Margulis, 1981; Maynard-Smith and Szathmáry, 1995). The emergence of integrated organisms has been investigated as the ''hypercycle'' of prebiotic evolution, in which each of the subunits can provide some adaptive benefit to its partner (Eigen and Schuster, 1979). However, because genetically unrelated organisms rarely start a mutualistic relationship but usually end up in parasitism (an exploiter–exploitee relationship) (Axelrod, 1984; Nowak and Sigmund, 1992, 1993), the evolution of cooperation should be viewed as having greater cooperative trait value than a threshold that provides something advantageous to its partner (Frank, 1995).

From an evolutionarily ecological point of view, mutualism is expected to originate from parasitism at the beginning. Two important factors have been targeted here: decrease in virulence and vertical transmission. As long as the host and the parasite coexist in a long-term relationship, parasites should evolve to decrease the level of exploitation and virulence on their host because too much exploitation and virulence would lead to extinction of the host and, consequently, the parasite itself. For example, *Myxoma* virus induced artificially into the rabbit

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population in Australia was highly lethal at first, but the virus evolved rapidly to lessen its virulence within a couple of decades, and the host and its parasites finally established coexistence without extinction (Fenner, 1965, 1983; Maynard-Smith, 1989). Levin and Pimentel (1981) and May and Anderson (1983) analyzed the rabbit—*Myxoma* coexistence theoretically.

Ewald (1987) compared transmission modes of parasites between horizontal and vertical and concluded that the fitness of parasites with vertical transmission should be reflected directly by the host's fitness and that such parasites are likely to exhibit low virulence. Herre (1993) showed that nematode parasites exhibit low virulence in a fig wasp species which has nearly vertical transmission.

Based on the early ESS model for the evolutionary shift from parasitism to mutualism (Roughgarden, 1975), Matsuda and Shimada (1993) and Yamamura (1993, 1996) showed that parasites could shift their transmission mode from horizontal to vertical. These models suggest that a mutualistic relationship can be selected for if the symbionts and their host can make use of metabolic wastes exerted by their partner (nonzero-sum game).

However, these ESS models were constructed to maximize particular strategic parameters reflecting the fitness (survivorship or reproduction), and none of the models considered metabolism as a whole within a cell. We took a novel approach by formulating a mathematical model that introduces the recently developed cell biology concept of "autophagy" (Levine and Klionsky, 2004; Lum et al., 2005; Mizushima, 2005). Almost all eukaryotic cells use the decomposing process of autophagy in intracellular metabolism, by which a cell recycles and renews the materials of its own organelles. The outline of this process is as follows. First, an autophagosome, a vesicle surrounded by a lipid membrane, is formed in the cytoplasm to enclose organelles or other structures or molecules. Next, the autophagosome fuses with the lysosome. Finally, the components in the cytoplasm are decomposed together with the autophagosome inside the lysosome (Yorimitsu and Klionsky, 2005). Empirical research also shows that the autophagic machinery can act as an innate defense system against invading pathogens (Nakagawa et al., 2004; Deretic, 2005; Schmid et al., 2005; Seay et al., 2006). Ogawa et al. (2005) found that an invader that can escape from autophagy can succeed as a parasite. It appears plausible that symbionts were, at the beginning of endosymbiosis, microbial invaders of their host cell and were able to be degraded by autophagy.

We incorporated the process of autophagic decomposing into the model of endosymbiosis by referring to and drawing on the idea of ecosystem ecology. In nature, the material cycle in the ecosystem is described on terms of the roles of the producer, the consumer, and the decomposer (Margalef, 1963; Odum, 1971, 1983; Ulanowicz, 1983). In natural ecosystems, plants that assimilate carbon and inorganic minerals using photoenergy act as the producer, animals grazing on plants act as the consumer, and fungi or bacteria reducing dead organic matter to nutrient minerals act as the decomposer. Lotka (1922) suggested that natural selection should tend to make the energy flow through the ecosystem a maximum; Loreau (1995) analyzed the impact of adding the consumer and concluded that consumption itself reinforces the turnover rate of material cycling when its consumption is moderate.

Similarly, the cell's metabolic system, consisting of biomaterial substances and enzymes, plays a self-production role in an autocatalytic way. Symbionts exploiting metabolites from a host cell are recognized as the consumer. Various cellular components, including the host cell's enzymes and symbionts, are decomposed by the autophagic process, which operates as the decomposer structurally or as the predator–recycler functionally (Andersen, 1997).

In this paper, we analyze the simultaneous ordinary differential equations (ODE) based on material cycling in the "cytological ecosystem", and we attempt to answer the following questions. First, what is the theoretical condition under which symbionts can exist in the host cell? Second, what determines the bifurcation between parasitism and mutualism? Finally, are there any common characteristics between endosymbiosis and ecosystem development?

2. Model and analysis

2.1. A free-living cell with no invader [Case 1]

Because a cell consists of a huge number of organic components and metabolic processes, it is difficult to describe all the elements and reactions in the metabolic pathways. We focus on low molecular weight organic substances in a cell, such as amino acids and sugars, to construct a simple scheme of metabolism, as illustrated in Fig. 1. This cell-metabolism model maintains metabolites N_1 and biomaterials N_2 by enzymes (E) that are synthesized from biomaterials N_2 themselves. Metabolites (N_1) correspond to metabolic intermediate substances to be transformed into other chemicals such as pyruvic acid or glucose-6-phosphate, which are used to produce amino acids or nucleotides. The model also includes biomaterials (N_2) existing as monomers, such as amino acids or nucleotides, which are used to synthesize functional polymers such as proteins or transfer RNAs.

For simplicity, we assume that catalytic reactions by enzymes take the first-order, linear interaction terms in this simultaneous ODE system. Because we are not interested in the quantitative behavior of the components in the system, the interactions are adopted as simply as possible, although we could set nonlinear forms as Michaelis–Menten reactions. As shown in Fig. 1, enzymes catalyze to produce N_2 from N_1 with efficiency a_1 and a_2 for the reverse reaction. Enzymes (E) are decomposed by autophagy at a constant rate d_1. Autophagosomes (A) are also assumed to be synthesized from biomaterials N_2 and to be destroyed by d_2. In reality, the synthesis of both E and A should be under genetic control, but we assume simply that they are made from N_2 at constant rates b_1 and b_2,

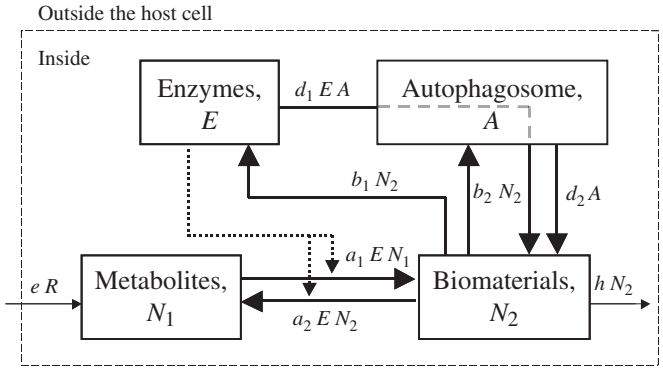

Fig. 1. Schematic representation of the material cycle of the model (Eqs. (1)–(4)). The flow of circulated materials is indicated by solid lines. A resource, denoted R, is provided with a constant rate e from outside the cell into this metabolic system. The amounts of metabolites and biomaterials are denoted by N_1 and N_2, respectively. The volumes of enzymes and autophagosome are represented as E and A, respectively, which are made from N_2 at constant rates b_1 and b_2, respectively. Metabolites and biomaterials are transformed bilaterally by the catalytic reaction by enzymes (dotted lines), where the efficiency of the reaction from metabolites to biomaterials is a_1, and a_2 for the reverse. Enzymes are decomposed by autophagy with a rate d_1, and their elements return to biomaterials. Autophagosomes themselves also decay with a rate d_2. Finally, biomaterials are used for growth with h outside the host cell.

respectively. The cell obtains the resource (R) from the surrounding environment outside the cell and converts it to metabolites N_1 with e and uses biomaterials N_2 at a rate h for growth of the cell itself.

We have assumed that the timescale is much shorter for the metabolic equilibrium state than for cell growth, and it gives coefficients e and h, which are much smaller than other reaction parameters. We focus on the maintenance of the cell metabolic system, which is presumed closed ($e \approx h \approx 0$).

Although the metabolic regulation is maintained by different enzymes in different pathways for each direction, we have combined these as one pathway to simplify the cell system, as depicted in Fig. 1.

Thus, the simultaneous ODE model reads:

$$\frac{dN_1}{dt} = -a_1 \cdot N_1 \cdot E + a_2 \cdot N_2 \cdot E, \tag{1}$$

$$\frac{dN_2}{dt} = a_1 \cdot N_1 \cdot E - a_2 \cdot N_2 \cdot E - b_1 \cdot N_2 - b_2 \cdot N_2 + d_1 \cdot E \cdot A + d_2 \cdot A, \tag{2}$$

$$\frac{dE}{dt} = b_1 \cdot N_2 - d_1 \cdot E \cdot A, \tag{3}$$

and

$$\frac{dA}{dt} = b_2 \cdot N_2 - d_2 \cdot A. \tag{4}$$

We also assume that the summation of all cellular elements is constant, Q, as the biomass at equilibrium. Therefore, Q is determined by the following equation:

$$N_1 + N_2 + E + A = Q. \tag{5}$$

To analyze the impact of endosymbiogenesis, we first consider the situation where the symbiont is absent. When this cell is closed ($e \approx h \approx 0$ in Fig. 1), all components in the host cell in model Eqs. (1)–(4) reach the following steady state (superscripted by an asterisk):

$$E^* = \frac{b_1}{d_1} \cdot \frac{d_2}{b_2}, \tag{6}$$

$$A^* = \frac{b_2}{d_2} \cdot N_2^*, \tag{7}$$

and

$$N_1^* = \frac{a_2}{a_1} \cdot N_2^*, \tag{8}$$

where

$$N_2^* = \frac{Q - E^*}{1 + a_2/a_1 + b_2/d_2}. \tag{9}$$

The intensity J_1 of the material cycling through biomaterials N_2 is measured by the inflow into or the outflow from N_2 because these flow intensities are balanced at the steady state. The inflow intensity is expressed as follows:

$$J_1 = a_1 \cdot N_1^* \cdot E^* + d_1 \cdot E^* \cdot A^* + d_2 \cdot A^*. \tag{10}$$

These results Eqs. (6)–(10) are used as the baseline and to compare with the metabolic systems, including the symbiont invasion, hereafter.

The steady state can be analyzed by the local stability condition by seeking the eigenvalue ($\forall Re(\lambda) < 0$) of the characteristic equation and shows the stability for this metabolic system (see Appendix A). This means that the metabolic system maintains homeostasis in the sense that the system returns to its steady state after perturbation caused by the variability of abiotic (e.g., temperature, etc.) and biotic (e.g., food resource) factors in the host cell.

2.2. With invaders (symbionts)

In this section, we address the essential issue that the host cell holds symbionts inside it. We call the symbionts within the host cell "invaders" regardless of the relationship they construct. The model is shown in Fig. 2 and is expressed mathematically as follows:

$$\frac{dN_1}{dt} = -a_1 \cdot N_1 \cdot E + a_2 \cdot N_2 \cdot E - f_1(N_1, P), \tag{1'}$$

$$\frac{dN_2}{dt} = a_1 \cdot N_1 \cdot E - a_2 \cdot N_2 \cdot E - b_1 \cdot N_2 - b_2 \cdot N_2 + d_1 \cdot E \cdot A + d_2 \cdot A - f_2(N_2, P) + d_3 \cdot P \cdot A, \tag{2'}$$

$$\frac{dE}{dt} = b_1 \cdot N_2 - d_1 \cdot E \cdot A, \tag{3'}$$

$$\frac{dA}{dt} = b_2 \cdot N_2 - d_2 \cdot A, \tag{4'}$$

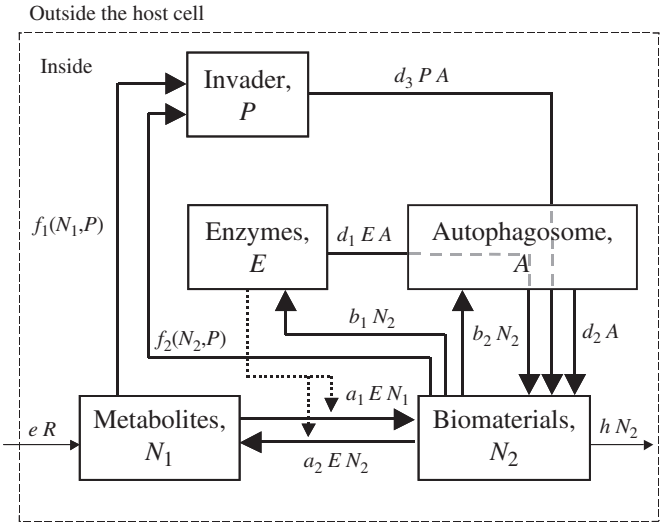

Fig. 2. The material cycle in the system including the invader (Eqs. (1′)−(4′) and (11)). The volume of the invader is denoted by P. The invader exploits metabolites N_1, in which the functional response is represented as $f_1(N_1, P)$, or biomaterials, represented as N_2 with $f_2(N_2, P)$. The autophagic process divests the invader at a rate d_3, and its elements return to biomaterials in its host cell.

and

$$\frac{dP}{dt} = f_1(N_1, P) + f_2(N_2, P) - d_3 \cdot P \cdot A. \tag{11}$$

The invaders (P) exploit metabolites N_1 or biomaterials N_2, or both, as the resource from the host cell with an interaction function $f_i(N_i, P)$. Using similar reasoning as in Case 1, we did not set nonlinear interactions for the functional response, and the first-order linear interactions are supposed simply as follows:

$$f_i(N_i, P) = r \cdot N_i \cdot P. \tag{12}$$

Invaders exploit several types of molecules from their host cell according to their needs. Instinctively, the invaders act parasitically for the first time because they exploit resources from their host cell as a necessity for survival instead of feeding by themselves. Autophagy works to defend against invaders by decomposing them at a constant rate d_3, and the components from invaders that are decomposed return to its host cell as biomaterials N_2.

In our model, we assume that the invaders exploit either metabolites N_1 or biomaterials N_2 from their host cell in two extremely simple cases. As in Case 1, we also assume that the summation of all components including the invaders is constant, Q, as the cell biomass at equilibrium as follows:

$$N_1 + N_2 + E + A + P = Q. \tag{13}$$

2.2.1. Invaders without metabolic assistance (exploiting only N_2) [Case 2]

First, let us consider Case 2 where invaders exploit only biomaterials N_2. In a mathematical sense, the interaction function $f_2(N_2, P)$ is effective, but $f_1(N_1, P)$ is omitted here. The elements in the model Eqs. (1′)−(4′) and (11) reach equilibrium at the following compartment sizes:

$$E^* = \frac{b_1}{d_1} \cdot \frac{d_2}{b_2}, \tag{14}$$

$$A^* = \frac{b_2}{d_2} \cdot N_2^*, \tag{15}$$

$$N_1^* = \frac{a_2}{a_1} \cdot N_2^*, \tag{16}$$

and

$$P^* = 0, \tag{17}$$

where

$$N_2^* = \frac{Q - E^*}{1 + a_2/a_1 + b_2/d_2}. \tag{18}$$

Because all components are the same as in Case 1, the invader cannot have a positive P^*, but this value is 0 and is excluded from this system. With respect to these solutions Eqs. (14)–(18), the stability given in Appendix B is implemented by the following inequation:

$$\frac{r}{d_3} < \frac{b_2}{d_2}. \tag{19}$$

The left side of inequation (19), the invader growth rate (r) divided by its mortality rate (d_3), indicates the relative increase in the rate of the invader. The right side gives the relative synthesis rate of autophagosomes in the same sense. Hence, the boundary stability ($P^* = 0$) is attained when the growth rate of the invader is lower than the synthesis rate of autophagosomes. We checked the dynamics numerically to determine when the invader can thrive faster than the host defense against it. As shown in Fig. 3(a), the host cell will no longer survive because it lacks N_2 absorbed by the invader. We have confirmed the existence of this dead stationary state, that is, the set of solutions $(N_1, N_2, E, A, P) = (0, 0, Q - P^*, 0, P^*)$ as illustrated in Fig. 3(a). We also checked the local stability of this state. Thus, the host cell could not keep the invaders stably in this metabolic system, and the relationship between the host and the invaders is regarded as parasitic or pathogenic.

2.2.2. Invaders with metabolic assistance (exploiting only N_1) [Case 3]

2.2.2.1. The coexistence condition and the local stability.

When we assume that the interaction between the host cell and its invaders becomes effective only in $f_1(N_1, P)$ but that $f_2(N_2, P)$ is invalid, this system will show two different steady states: one is the same as the previous case (parasitism) and the other is quite different (emergence of mutualism). In the original case of this system at equilibrium, the compartment sizes are expressed as follows:

$$E^* = \frac{b_1}{d_1} \cdot \frac{d_2}{b_2}, \tag{20}$$

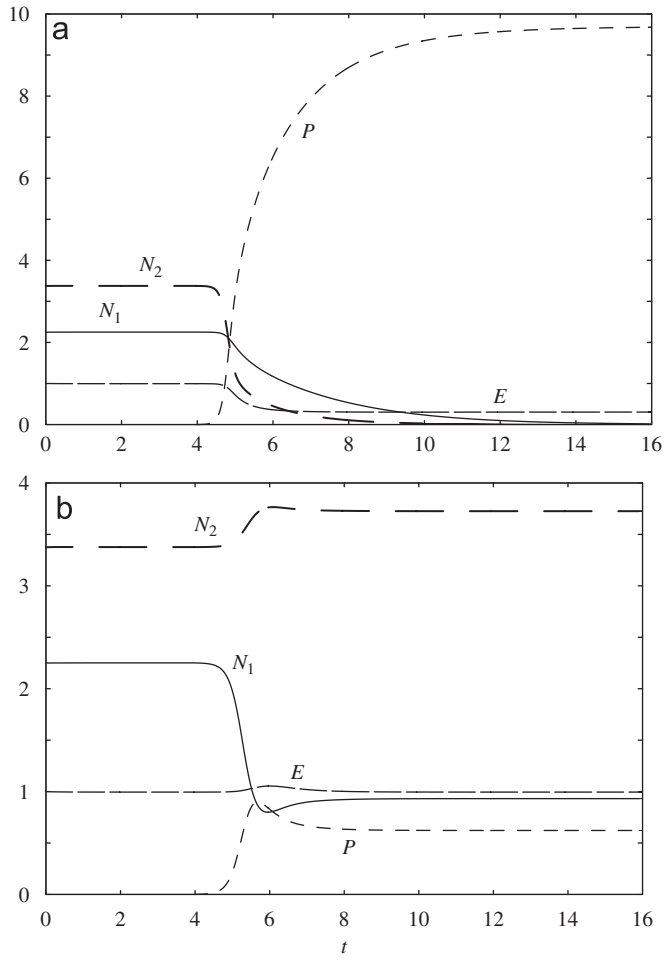

Fig. 3. An example of the metabolic dynamics within the host cell. All components are in the steady state; there is no invader at the beginning, but the invader is induced at $t = 4.0$. (a) The invader exploits biomaterials N_2 and grows faster than the autophagosome is synthesized. The population size P increases until the resource N_2 is depleted. (b) This is the case when the invader exploits N_1 as the resource and can coexist within the host cell. The invader unspreads and the population dynamics maintains the steady state quickly. Parameter values: $a_1 = 1.5$, $a_2 = b_1 = b_2 = d_1 = d_2 = d_3 = 1.0$, $r = 4.0$, the total biomass $Q = 10.0$. The initial population size of the invader $P_{ini} = 0.001$.

$$A^* = \frac{b_2}{d_2} \cdot N_2^*, \tag{21}$$

$$N_1^* = \frac{d_3}{r} \cdot \frac{b_2}{d_2} \cdot N_2^*, \tag{22}$$

and

$$P^* = \frac{b_1}{d_1} \cdot \frac{d_2}{b_2} \cdot \frac{a_2}{r} \left(\frac{r}{d_3} \cdot \frac{d_2}{b_2} - \frac{a_1}{a_2} \right), \tag{23}$$

where

$$N_2^* = \frac{Q - E^* - P^*}{1 + d_3/r \cdot b_2/d_2 + b_2/d_2}. \tag{24}$$

Considering Eq. (23), the following inequation is required to have a positive solution for the invaders P^*:

$$\frac{r}{d_3} > \frac{a_1}{a_2} \cdot \frac{b_2}{d_2}. \tag{25}$$

In the same way as the inequation (19), the invaders at equilibrium (P^*) can persist in the system if, and only if, their relative growth rate (r/d_3) is greater than the product of the relative synthesis rate of autophagosomes and the productivity of N_2 by the host itself ($a_1/a_2 \cdot b_2/d_2$). Meeting the inequation (25) is necessarily and sufficiently required for the stability of this steady state (details are given in Appendix C). Numerical simulation proves that this system is stable both locally and globally; this is illustrated in Fig. 3(b).

We can express the condition of the boundary stability in Case 3 where the invaders will not be able to coexist but are eliminated when they exploit N_1:

$$\frac{r}{d_3} < \frac{a_1}{a_2} \cdot \frac{b_2}{d_2}. \tag{26}$$

As the reverse of inequation (25), inequation (26) generates $P^* = 0$. We analyzed the necessary condition for the boundary stability at $P^* = 0$ similarly to the analysis for Case 2. Meeting the inequation (25) results in $P^* > 0$, indicating the necessary condition for coexistence between the host and symbiont.

2.2.2.2. Threshold discriminating between parasitism and mutualism. Considering the decomposition of invaders, the intensity J_2 of the material cycling is expressed as follows:

$$J_2 = a_1 \cdot N_1^* \cdot E^* + d_1 \cdot E^* \cdot A^* + d_2 \cdot A^* + d_3 \cdot P^* \cdot A^*. \tag{27}$$

Then, the difference in the cycling intensity between the two cases whether the invaders could exist or not ($\delta J = J_2 - J_1$) is calculated as follows:

$$\delta J = J_2 - J_1 = \frac{(a_2/a_1 - d_3/r \cdot b_2/d_2)}{(1 + b_2/d_2 + d_3/r \cdot b_2/d_2)(1 + a_2/a_1 + b_2/d_2)} \times (Q - Q'), \tag{28}$$

where

$$Q' = ((a_1 + a_2)\frac{d_2}{b_2} + (a_1 + d_3))\frac{E^*}{d_3}. \tag{29}$$

The flow intensity J increases or decreases depending on the threshold value Q' because the numerator of the first term in Eq. (28) is positive when the condition of the inequation (25) is satisfied.

Case 3 has two different parametric values concerning Q under the same threshold Q'. The curve of J as a function of r is illustrated in Fig. 4. As expected from the calculations given above in Eqs. (28) and (29), for $Q > Q'$ (Fig. 4(a)), J increases monotonically with increasing r,

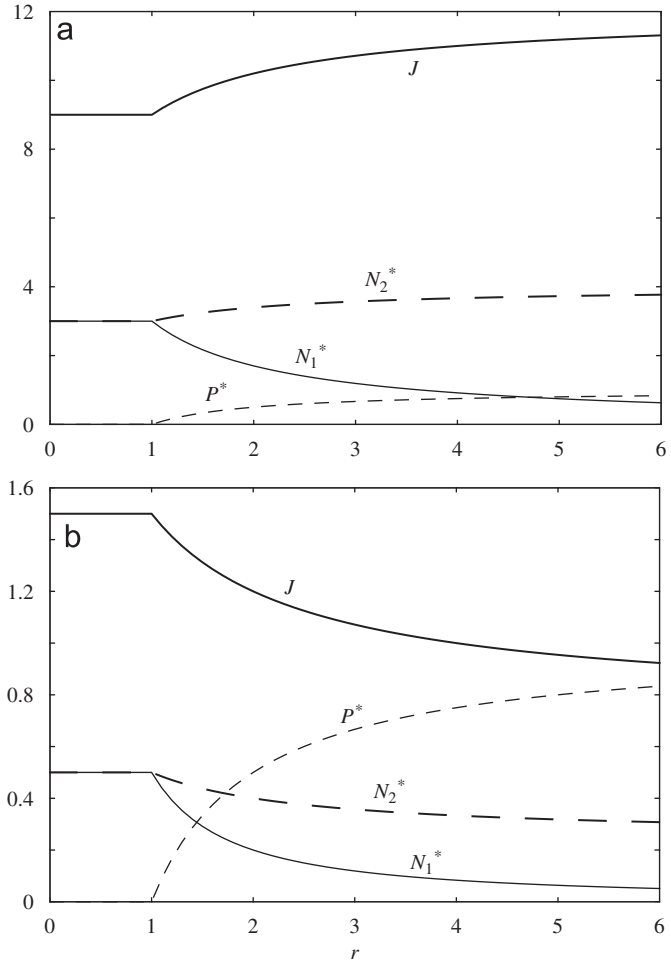

Fig. 4. Material cycling flow (J) and the compartment sizes in equilibrium as a function of the growth rate of the symbiont, r, when the symbiont assists its host with effective metabolic advantage. (a) The total biomass is larger than the threshold, and the host–symbiont relation should be mutualistic. (b) The total biomass is smaller, and the relation can still be parasitic. Parameter values: $a_1 = a_2 = b_1 = b_2 = d_1 = d_2 = d_3 = 1.0$, the threshold value $Q' = 4.0$ in both cases; total biomass $Q = 10.0$ in (a), and $Q = 2.5$ in (b).

whereas for $Q < Q'$ (Fig. 4(b)), J decreases. Because the value Q can correspond to the total amount of host cell components (is equal to the total host cell size), we conclude that evolution to either parasitism or mutualism depends on the host cell size. Metabolites N_2 follow the same two distinct patterns as J with increasing r.

These tendencies are proved by their partial derivatives in respect to r as follows:

$$\frac{\partial J_2}{\partial r} = \frac{b_1 + b_2 + d_3\,(b_2/d_2)P^*}{(1 + a_2/a_1 + b_2/d_2)^2 \cdot r^2} \cdot (Q - Q'), \tag{30}$$

$$\frac{\partial N_2^*}{\partial r} = \frac{d_3 \cdot b_2/d_2}{(1 + a_2/a_1 + b_2/d_2)^2 \cdot r^2} \cdot (Q - Q'), \tag{31}$$

$$\frac{\partial N_1^*}{\partial r} = -\frac{(Q - E - P^*)}{r} - P^*(1 + b_2/d_2 + d_3/r \cdot b_2/d_2) < 0, \tag{32}$$

and

$$\frac{\partial P^*}{\partial r} = \frac{a_1}{r^2} \cdot \frac{b_1}{d_1} \cdot \frac{d_2}{b_2} > 0. \tag{33}$$

Thus, the equilibrium sizes of N_1^* and P^* do not depend on the threshold Q': P^* always increasing with higher r and N_1^* decreasing, provided that the inequation (25) is satisfied. That is, the more P^* increases, the more N_1 is exploited. The reverse, to meet the inequation (26), indicates the extinction of P^* where $0 < r < 1$ in Fig. 4. Despite the increasing trend of P^* with respect to r, the maximum equilibrium size should be limited by N_1. Thus, the population size of the invaders is not determined by its own growth rate but depends on the host parameter sets.

3. Discussion

3.1. The condition for coexistence

We demonstrated mathematically the conditions determining whether invaders can coexist within their host cell. Invaders that do not assist their host through exploiting N_1 metabolically cannot establish a stable coexistence in equilibrium. Such invaders will either be excluded by their host's autophagy or burst out of the host cell as parasites or pathogens. In the former, the host cell can digest the invaders if the synthesis rate of autophagosomes is greater than the invader's growth rate. Otherwise, invaders persist inside the host cell if their metabolic performance is better than the host's or if their growth rate is high enough to escape from autophagy.

For example, at the evolutionary beginning of the eukaryotic cell a couple of billion years ago, ancestors of the mitochondrion were likely to have had a higher ATP productivity than the ancestral pre-eukaryotic host cells (Andersson et al., 2003; John and Whatley, 1975). In the case of the insect, the benefit to the host insect through its symbiotic bacteria originates from the bacterial product (Ishikawa, 1985; Bourtzis and Miller, 2006), which the host cannot synthesize by itself. This situation corresponds to the low performance of catalytic activity for N_1 to N_2 reaction by enzymes or the lack of enzymes in the host cell in our model, where it is easier to establish a symbiotic relationship between the host and its invader because the threshold, Q', is quite low. In other words, this resembles a process of complete niche differentiation between the enzymes and the invader.

Our assumption that the invader exploits either N_1 or N_2 from the host is extremely simple and may be far from the reality of nature. However, the metabolic assistance from the invader to its host cell should be indispensable for generating the mutualistic symbiosis relationship regardless of how the invader exploits particular substances from its host. From the ecological perspective, the coexistence cannot be maintained and the competitive exclusion should occur without differentiating the invader from the autophagosome and its niche.

3.2. Evolution to parasitism or mutualism?

Once the invader enters the host cell and a new combination of metabolic cycles starts, one may ask whether the new metabolic system is advantageous. As illustrated in Fig. 4, the invader numbers (P) increase by increasing the growth rate (r) monotonically, and the metabolite N_1 decreases because of exploitation of N_1 by the invaders as the resource, and vice versa. The transition into biomaterials (N_2) depends on the host biomass Q, which corresponds to the host cell size. When the host cell has a large biomass ($Q > Q'$), N_2 increases with increasing r. In the case with a large cell biomass, the host cell can suppress its symbionts adequately and return to N_2 efficiently, and this results in abundant production of N_2. Thus, endosymbiosis promotes mutualistic evolution to produce a combination that benefits the organism.

Viewed from a different angle, the resilience of this metabolism should strengthen in sync with the enhancement of the material flow along with an increase in r. It is plausible that the host cells cannot feed their resource constantly under a changing environment. In such a situation, the metabolic system which is disordered seriously depending upon the surrounding is not survive, hence the cell must adjust its own state quickly. Therefore, it is conceivable that the enhancement of the material flow inside the cell, which increases the resilience, is advantageous for the cell. In the natural ecosystem, resilience is an important concept because of its stability against environmental perturbation (Pimm, 1991); the relation between the resilience and the flow through the system has been discussed previously (DeAngelis, 1992).

On the other hand, N_2 decreases gradually if the host cell biomass is smaller than the threshold Q' ($Q < Q'$). Autophagosomes may not be synthesized sufficiently in a small cell, and the symbionts are not depressed severely by the host's autophagosomes. Therefore, the decrease in N_2 delays the cell's growth, and this delay turns to disadvantage for the host cell's fitness. In other words, the symbionts will be "parasites" here.

The present study differs completely from previous symbiosis models that have studied the dynamics of coevolution between an infectious disease agent and its host, leading to the balance or the arms race between parasite attacks and host defenses (Frank, 1993; van Baalen, 1998; Sasaki and Godfray, 1999). In contrast, our model is based on autophagy, the universal process of eukaryotes in which any host can use its immune system to defend against pathogens or parasites. Our conclusions can also be applied to an organism that does not have a highly developed immune system such as a single-cell organism as the host.

Our model leads to a new scenario in which the mutualistic relationship originates from the efficient metabolic assistance by the symbiont but does not originate from any decrease in exploitation, as suggested by Roughgarden (1975) and Yamamura (1993). The parasites that exploit only their host without giving any assistance to it never participate in mutualistic symbiosis in our model. In addition, for the emergence of mutualism, our model requires the host to have a large cell size initially, which is coincident with Frank's (1995) claim that a pair of organisms can start mutualistic evolution if they have a value of cooperative trait greater than the threshold at the beginning.

3.3. The common properties between a metabolic system and the ecosystem

At the ecosystem level in nature, consumers contribute to the system when they have a growth rate greater than some threshold, and more importantly, the material cycling flow is accelerated by adding the consumers (Loreau, 1995). Otsuka (2004) showed a similar pattern when the ecosystem is complicated by adding carnivores to increase the number of trophic levels. This tendency is recognized widely in theoretical ecosystem models (Odum, 1969; Loreau, 1998). The reason why material cycling accelerates is that decomposers such as bacteria can decompose dead animal bodies faster than they can decompose dead leaves. Also, Loreau (1995) claimed that the total quantity of nutrients in the ecosystem must be higher than some threshold value when the consumer enhances the circular flow.

Even though the cytological metabolic system in which autophagosomes decompose elements in a cell is constructed in a different way from the natural ecosystem, we note that the two systems share some common characteristics: (i) symbionts as consumers are required to have a higher growth rate than the rate of autophagosome decomposition, (ii) the host cell must be larger than a certain threshold size to establish a mutualistic relationship with its symbiont, and (iii) this partnership accelerates the biomaterial turnover flow.

The present results are based on steady-state analysis of the systems and, as a result, we have several subjects to address in future. First, the effects of nonlinear interactions of the functional response must be taken into account to focus on the evolution from parasitism to mutualism because these strongly influence the host–parasite dynamics (Hassell, 1978). Second, from an evolutionary perspective, we should examine whether symbiont-containing cells as "mutants" can spread into a free-living host-cell population. Numerical simulations will provide insights into the evolutionary population dynamics of the host–symbiont system.

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Appendix A. The local stability in Case 1

The local stability of the Case 1 system described by Eqs. (1)–(4) can be analyzed proving that the eigenvalues of its Jacobian matrix are negative for their real parts.

We can reduce the rank of the Jacobian matrix about the simultaneous equations (1)–(4), because Eq. (2) consists of the linear combination of the other equations. The local stability of the subsystem at equilibrium is then determined by the eigenvalues of its Jacobian matrix as follows:

$$A=\begin{bmatrix} -a_1\cdot E^* & -a_1\cdot N_1^*+a_2\cdot N_2^* & 0\\ 0 & -d_1\cdot A^* & -d_1\cdot E^*\\ 0 & 0 & -d_2 \end{bmatrix}. \tag{A.1}$$

Then, setting the eigenvalues as λ's, we derive the characteristic equation from this Jacobian matrix (A.1) like

$$(\lambda+a_1\cdot E^*)(\lambda+d_1\cdot A^*)(\lambda+d_2)=0. \tag{A.2}$$

Obviously, the eigenvalues are negative because all coefficients are positive, and this system maintains the steady state locally.

We can also connect the idea of stability to flux intensity (J_1) expressed by Eq. (10). The resilience, one index of system stability, is measured by the inverse of the dominant eigenvalue. If we consider Eq. (10), the flux intensity (J_1) consists of three terms, each of which includes the three eigenvalues. Therefore, we can expect a positive correlation between the resilience of the system and the flux intensity.

In addition, let us consider the case where the metabolic system is not closed completely ($e\approx h>0$), but the system keeps its total biomass over the metabolic reaction time-scale ($e\cdot R=h\cdot N_2^*$). Although we need to reformulate the ODE to include the terms ($e\cdot R$) into Eq. (1) and ($-h\cdot N_2^*$) into Eq. (2), the Jacobian matrix is the same as (A.1), and the analysis of the local stability obtains the same result as that in the closed one.

Appendix B. The local stability in Case 2

The stability of the system described by Eqs. (1′)−(4′) and (11) can be analyzed in the same manner as in Case 1. First, let us consider the case where the invader does not assist the host at all, that is, the solutions of this system in (14)–(18).

We can derive the Jacobian matrix of the system to include parasites as follows:

$$A=\begin{bmatrix} -a_1\cdot E^* & -a_1\cdot N_1^*+a_2\cdot N_2^* & 0 & 0\\ 0 & -d_1\cdot A^* & -d_1\cdot E^* & 0\\ 0 & 0 & -d_2 & 0\\ 0 & 0 & -d_3\cdot P^* & -d_3\cdot A^*+r\cdot N_2^* \end{bmatrix}. \tag{B.1}$$

Therefore, we obtain the characteristic equation as follows:

$$(\lambda+a_1\cdot E^*)(\lambda+d_1\cdot A^*)(\lambda+d_2)(\lambda+d_3\cdot A^*-r\cdot N_2^*)=0. \tag{B.2}$$

The local stability requires the term ($d_3\cdot A^*-r\cdot N_2^*$) to be positive. Assigning parameters (15) to the equilibrium solution A^*, we can easily obtain the necessary and sufficient condition for local stability (the inequation (19)).

Appendix C. The local stability in Case 3

There are two different states when the invader exploits N_1, meaning that the solutions are expressed as either (14)–(18) or (20)–(24). To check the local stability for each case, we begin by deriving the Jacobian matrix of the system.

$$A=\begin{bmatrix} -a_1\cdot E^*-r\cdot P^* & -a_1\cdot N_1^*+a_2\cdot N_2^* & 0 & -r\cdot N_1^*\\ 0 & -d_1\cdot A^* & -d_1\cdot E^* & 0\\ 0 & 0 & -d_2 & 0\\ r\cdot P^* & 0 & -d_3\cdot P^* & -d_3\cdot A^*+r\cdot N_1^* \end{bmatrix}. \tag{C.1}$$

We obtain the characteristic equation as follows:

$$(\lambda+d_1\cdot A^*)(\lambda+d_2)\{(\lambda+a_1\cdot E^*+r\cdot P^*)\times(\lambda+d_3\cdot A^*-r\cdot N_1^*)+r^2\cdot N_1^*\cdot P^*\}=0. \tag{C.2}$$

The characteristic equation can be reduced into three terms; the first and second terms show that the eigenvalues are negative. The local stability is, therefore, determined according whether the last term can have a negative eigenvalue.

First, let us consider the case when the invader can coexist with the host. Assigning the parameters to the equilibrium solutions except for N_2, which is given as (20)–(23), the last quadratic equation is expressed as follows:

$$\lambda^2+a_2\cdot\frac{b_1}{d_1}\cdot\left(\frac{d_2}{b_2}\right)^2\cdot\frac{r}{d_3}\cdot\lambda+\frac{b_1}{d_1}\cdot\frac{d_2}{b_2}\cdot a_2\cdot d_3\cdot N_2^*\left(\frac{r}{d_3}-\frac{a_1}{a_2}\cdot\frac{b_2}{d_2}\right)=0. \tag{C.3}$$

If the coefficients are positive for the quadratic equation, $Re(\lambda)$ becomes negative, and the last term in (C.3) must be positive to assure the local stability determined by the Routh–Hurwitz criteria (Gantmacher, 1960). Therefore, the condition for the local stability is equivalent to meet the inequation (25).

Furthermore, we can refer to an effect of the symbiont's growth rate on the system's local stability, and, in turn, on the resilience of the metabolic system. As the growth rate r increases, at the steady state the invader's population (P^*) also increases within the host cell, which should increase the flow intensity (J). Considering the characteristic equation (C.3), the increase in r has an important implication for the system's resilience. The absolute value of the solution of Eq. (C.3) changes in proportion to the

coefficient of the second term. Then, as the parameter r increases, the eigenvalue becomes the dominant one, and the system should return to its steady state more quickly after environmental perturbation. In other words, if the symbionts grow faster in the host cell, the metabolic system of the host cell results in a more resilient one.

Second, we can derive the condition for boundary stability at $P^* = 0$. The quadratic equation which assigns $P^* = 0$ as the parameters given as (14)–(18) is as follows:

$$(\lambda + a_1 \cdot E^*)(\lambda + d_1 \cdot A^*)(\lambda + d_2)(\lambda + d_3 \cdot A^* - r \cdot N_1^*) = 0. \tag{C.4}$$

Local stability requires the term $(d_3 \cdot A^* - r \cdot N_1^*)$ to be positive. By assigning parameters given as (15) and (16) to the equilibrium solutions, we can easily show that the condition for local stability is synonymous with meeting the inequation (26).

References

Andersen, T., 1997. Pelagic Nutrient Cycles: Herbivores As Sources and Sinks. Springer, Berlin.

Andersson, S., Karlberg, O., Canback, B., Kurland, C., 2003. On the origin of mitochondria: genomics perspective. Philos. Trans. R. Soc. B 358 (1429), 165–177.

Axelrod, R., 1984. The Evolution of Cooperation. Basic Books, New York.

Bourtzis, K., Miller, T.A., 2006. Insect Symbiosis. CRC Press, Boca Raton, FL.

Buchner, P., 1965. Endosymbiosis of Animals with Plant Microorganisms. Wiley Interscience, New York.

DeAngelis, D.L., 1992. Dynamics of Nutrient Cycling and Food Webs. Chapman & Hall, London.

Deretic, V., 2005. Autophagy in innate and adaptive immunity. Trends, Immunol. 26 (10), 523–528.

Eigen, M., Schuster, P., 1979. The Hypercycle: a Principle of Natural Self-organization. Springer, Berlin.

Ewald, P.W., 1987. Transmission modes of evolution of the parasitism-mutualism continuum. Ann. N.Y. Acad. Soc. 503 (1), 295–306.

Fenner, F., 1965. Myxoma virus and *Oryctolagus cuniculus*. In: Baker, H., Stebbins, A. (Eds.), The Genetics of Colonizing Species. Academic Press, New York, pp. 485–501.

Fenner, F., 1983. The Florey lecture, 1983. Biological-control, as exemplified by smallpox eradication and myxomatosis. Proc. R. Soc. London B 218, 259–285.

Frank, S.A., 1993. Evolution of host–parasite diversity. Evolution 47 (6), 1721–1732.

Frank, S.A., 1995. The origin of synergistic symbiosis. J. Theor. Biol. 176 (3), 403–410.

Gantmacher, F., 1960. The Theory of Matrices, vol. 1. Chelsea Publisher, New York.

Hassell, M.P., 1978. The Dynamics of Arthropod Predator–Prey Systems. Princeton University Press, Princeton, NJ.

Herre, E.A., 1993. Population structure and the evolution of virulence in nematode parasites of fig wasps. Science 259 (5100), 1142–1445.

Ishikawa, H., 1985. Symbionin, an aphid endosymbiont-specific protein. I: production of insects deficient in symbiont. Insect Biochemistry 15, 155–163.

John, P., Whatley, F., 1975. Paracoccus denitrificans and the evolutionary origin of mitochondria. Nature 254, 495–498.

Levin, S., Pimentel, D., 1981. Selection of intermediate rates of increase in parasite–host systems. Am. Nat. 117 (3), 308–315.

Levine, B., Klionsky, D.J., 2004. Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev. Cell 6 (4), 463–477.

Loreau, M., 1995. Consumers as maximizers of matter and energy flow in ecosystems. Am. Nat. 145 (1), 22–42.

Loreau, M., 1998. Ecosystem development explained by competition within and between material cycles. Proc. R. Soc. London B 265 (1390), 33–38.

Lotka, A., 1922. Contribution to the energetics of evolution. Proc. Natl Acad. Sci. USA 8 (6), 147–151.

Lum, J., DeBerardins, R., Thompson, C., 2005. Autophagy in metazoans: cell survival in the land of plenty. Nat. Rev. Mol. Cell Biol. 6 (6), 439–448.

Margalef, R., 1963. On certain unifying principles in ecology. Am. Nat. 97 (897), 357–374.

Margulis, L., 1981. Symbiosis in Cell Evolution. W.H. Freeman, New York.

Matsuda, H., Shimada, M., 1993. Cost-benefit model for the evolution of symbiosis. In: Kawanabe, H., Cohhen, E., Iwasaki, K. (Eds.), Mutualism and Community Organization. Behavioural, Theoretical, and Food-Web Approaches. Oxford, UK, pp. 228–238.

May, R.M., Anderson, R.M., 1983. Parasite–host coevolution. In: Futuyma, D., Slatkin, M. (Eds.), Coevolution. Sinauer Associates Inc, New York, pp. 186–206.

Maynard-Smith, J., 1989. Evolution—generating novelty by symbiosis. Nature 341 (6240), 284–285.

Maynard-Smith, J., Szathmáry, E., 1995. The Major Transitions in Evolution. W.H. Freeman, Oxford.

Mizushima, N., 2005. The pleiotropic role of autophagy: from protein metabolism to bactericide. Cell Death Differ. 12, 1535–1541.

Nakagawa, I., Amano, A., Mizushima, N., Yamamoto, A., Yamaguchi, H., Kamimoto, T., Nara, A., Funao, J., Nakata, M., Tsuda, K., Hamada, S., Yoshimori, T., 2004. Autophagy defends cells against invading group a streptococcus. Science 306 (5698), 1037–1040.

Nowak, M.A., Sigmund, K., 1992. Tit for tat in heterogeneous populations. Nature 355, 250–253.

Nowak, M.A., Sigmund, K., 1993. A strategy of win-stay, lose-shift that outperforms tit-for-tat in the prisoner's dilemma game. Nature 364, 56–58.

Odum, E.P., 1969. The strategy of ecosystem development. Science 164, 262–270.

Odum, E.P., 1971. Fundamentals of Ecology, third ed. W B Saunders Co, New York.

Odum, H.T., 1983. Systems Ecology. Wiley, New York.

Ogawa, M., Yoshimori, T., Suzuki, T., Sagara, H., Mizushima, N., Sasakawa, C., 2005. Escape of intracellular shigella from autophagy. Science 307 (5710), 727–731.

Otsuka, J., 2004. A theoretical characterization of ecological systems by circular flow of materials. Ecol. Complexity 1 (3), 237–252.

Pimm, S.L., 1991. The Balance of Nature?: Ecological Issues in the Conservation of Species and Communities. University of Chicago Press, Chicago.

Roughgarden, J., 1975. Evolution of marine symbiosis: a simple cost-benefit model. Ecology 56 (5), 1201–1208.

Sasaki, A., Godfray, H., 1999. A model for the coevolution of resistance and virulence in coupled host–parasitoid interactions. Proc. R. Soc. London B 266 (1418), 1455–1463.

Schmid, D., Dengjel, J., Schoor, O., Stevanovic, S., Munz, C., 2005. Autophagy in innate and adaptive immunity against intracellular pathogens. J. Mol. Med. 84 (3), 194–202.

Seay, M., Patel, S., Dinesh-Kumar, S., 2006. Autophagy and plant innate immunity. Cell. Microbiol. 8 (6), 899–906.

Ulanowicz, R., 1983. Identifying the structure of cycling ecosystems. Math. Biosci. 65 (2), 210–237.

van Baalen, M., 1998. Coevolution of recovery ability and virulence. Proc. R. Soc. London B 265 (1393), 317–325.

Yamamura, N., 1993. Vertical transmission and evolution of mutualism from parasitism. Theor. Popul. Biol. 44, 95–109.

Yamamura, N., 1996. Evolution of mutualistic symbiosis: a differential equation model. Res. Popul. Ecol. 38 (2), 211–218.

Yorimitsu, T., Klionsky, D., 2005. Autophagy: molecular machinery for self-eating. Cell Death Differ. 12, 1542–1552.