Theoretical study of evolutionary emergence and maintenance of mutualistic symbiosis

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Summary

Mutualism based on reciprocal exchange of costly services must avoid exploitation by "free-rides". Previous studies in the field of evolutionary ecology have explored vigorously how mutualism is evolutionarily maintained, and proposed various mechanisms to explain it. However, the evolution of symbiotic relationship is still unclear because it remains to be solved how the maintenance mechanisms themselves evolve. Since these mechanisms are essential to mutualism, their evolution is a central question to be investigated for understanding the evolution of symbiosis, in particular how symbiosis evolves toward not parasitism but mutualism. Here I theoretically investigated the evolution of maintenance mechanisms of mutualism. Mechanisms maintaining mutualism are usually classified into two types: "discrimination", which is an active behavioral response to the quality or behavior of partners to reward only cooperators; and "partner fidelity feedback", which is an automatic positive feedback between the fitness of participants involved in symbiosis. I therefore dealt with both types of mechanisms.

In chapter 2, I first focused on discrimination. In mutualism where a host acquires new symbionts from its environment, or horizontally transmitted mutualism, the host usually discriminates against free-riding symbionts. A well-known example is legumes that penalize non-cooperative rhizobia by halting oxygen and nutrient supply to them. Although discrimination promotes the evolution of cooperation, the evolution of discrimination has been considered to be difficult; once discrimination by hosts effectively removes free-riders from the symbiont population and cooperators become prevalent, a host can almost always meet cooperators and hence no longer needs to

discriminate among symbionts. In other words, it has been argued that discrimination and thus mutualism cannot be maintained unless free-rides are supplied perpetually by mutation and/or immigration. In this chapter, I tried to resolve the "paradoxical" coevolution of discrimination by hosts and cooperation by symbionts, by comparing two different types of discrimination: "one-shot" discrimination, where a host does not reacquire new symbionts after evicting free-riders, and "resampling" discrimination, where a host does from the environment. My study shows that this apparently minor difference in discrimination types leads to qualitatively different evolutionary outcomes. First, although it has been usually considered that the benefit of discriminators is derived from the variability of symbiont quality, I showed that the benefit of a certain type of discriminators (e.g. one-shot discrimination) is proportional to the frequency of free-riders, which is in stark contrast to the case of resampling discrimination. As a result, one-shot discriminators can invade the free-rider/non-discriminator population, even if standing variation for symbiont quality is absent. Second, my one-shot discriminators can also be maintained without exogenous supply of free-riders and hence is free from the paradox of discrimination. Therefore, my result indicates that the paradox is not a common feature of evolution of discrimination but is a problem of specific types of discrimination.

In chapter 3, I focused on partner fidelity feedback. In mutualism between unicellular hosts and their endosymbionts, symbiont cell division is often synchronized with its host's, ensuring its secure vertical transmission. Synchronized cell division can therefore align the fitness interests of hosts and symbionts and be a driving force of partner fidelity feedback. However, if symbionts stopped synchronizing and divided faster than their host, they could burst the host cell and could proliferate more

effectively via horizontal transmission. Therefore, symbionts face the tradeoff between efficient vertical transmission through self-restrained division and efficient horizontal transmission through rapid proliferation within a host. Here, I theoretically explored the condition for the evolution of self-restrained symbiont division. I assumed that symbionts control their division rate and that hosts control symbionts' death rate by intracellular digestion. In particular, I assumed the following: symbiosis helps both hosts and symbionts to survive; when a host cell divides, its daughter cells inherit its symbionts randomly; when a symbiont divides in a host cell, the divided cells accumulate in the host cell and eventually leads to its burst. My analysis shows that symbionts decrease their cell division rate evolutionarily if not only symbiont's but also host's benefit through symbiosis is large. Moreover, two outcomes arose as evolutionary bistability: the coevolution of hosts and symbionts leads to either secure symbiosis where symbionts is vertically transmitted through synchronized cell division, or the arms race where symbionts behave as lytic parasites and hosts resist by digesting them.

In chapter 4, as future perspectives of studies on the evolution of symbiosis, I discussed the evolutionary transition from free-living to organelle. During the evolutionary transition, the maintenance mechanism of mutualism probably switches from discrimination to vertical transmission (partner fidelity feedback), or the two mechanisms become to work together. Thus, the coevolution of discrimination and vertical transmission is an important problem to investigate the evolutionary transition. Although I did not analyze the coevolutionary dynamics directly, my above results provide an insight into the coevolution of discrimination and vertical transmission and the evolutionary emergence of organelle.

Contents

1. Introduction

1.1. The world shaped by symbiosis

Any organism lives not alone but together with others. *Symbiosis* (“living together” in Greek) is a close association between species that is permanent, long lasting, or frequent (Paracer & Ahmadjian, 2000). Symbiosis can be observed everywhere and spread into every domain and kingdom. Inside of our body, many bacteria in the gut help us to digest foods. Moreover, a ruminant keeps not only bacteria but also archaea in its rumen. In a garden, insects and birds pollinate flowering plants, and on the plants, ants protect and keep aphids. Even in sterile and dry soils, land plants can obtain nutrients and water from root nodule bacteria and mycorrhizal fungi. Alliances between fungi and algae, called lichen, can cover tundra. In the tropical ocean, a coral keeps algae for photosynthesis. On the coral, a wrasse cleans the mouth of groupers by eating parasitic isopods, which live in the mouth of the client and suck in their blood. In the open sea, where nutrients are limited, a kind of protozoa can live by carrying and keeping cyanobacteria. Diving into the deep sea, there is a different world where creatures live with chemosynthetic bacteria. Furthermore, all of these organisms are targeted by viruses.

Symbiosis also plays a critical role for the emergence and adaptive radiation of the biotic world found today. A genome is an aggregation of “symbiotic” genes that have different origins from each other (Maynard Smith & Szathmáry, 1993, 1995; Frank, 1994, 1997). Eukaryotic cells emerged by obtaining mitochondria and plastids, which are derived from symbiotic bacteria (Mereschkowsky, 1905; Margulis, 1981; Martin & Kowallik, 1999). Sex might have emerged in order to resist symbiotic enemies, namely

pathogens and parasites, by evolving faster than them (Hamilton, 1980; Hamilton *et al.*, 1990). As introduced above, many organisms advanced to new severe environments by relying on symbiotic partners. In particular, plants were not able to colonize on land without the help by symbiotic fungi (Selosse & Le Tacon, 1998), and a hydrothermal vent should be a barren zone unless animals relate with chemoautotrophic bacteria (Cavanaugh *et al.*, 2006; Dubilier *et al.*, 2008). Pollination generates highly diversified angiosperms (Armbruster, 2014), and the symbiotic interaction between corals and algae is a cradle of the huge diversity in the tropical ocean (Davy *et al.*, 2012). Based on these observations, there should be no doubt that symbiosis has played a central role in both the ecology and the evolution of life on Earth (Herre *et al.*, 1999; Paracer & Ahmadjian, 2000; Bronstein, 2015).

1.2. Evolution of symbiotic relationship: to help or to exploit?

As above examples, organisms involved in symbiosis can usually be benefited or exploited through the interactions. Depending on whether the relationship is beneficial or harmful, various types of symbiosis are usually classified as follows (Paracer & Ahmadjian, 2000; Bronstein, 2009, 2015): “mutualism” in which both involved partners benefit from each other; “commensalism” in which one benefits and the other is neither harmed nor benefited; “parasitism” in which one benefits by exploiting the other and thus the exploited one is harmed. Note that these terminologies, especially mutualism and symbiosis, are inconsistent among researchers (Paracer & Ahmadjian, 2000; West *et al.*, 2007; Bronstein, 2015) and that in this thesis, I obey one of the common definitions where both mutualism and parasitism are treated as subdivisions of symbiosis. In addition, I call organisms involved in symbiosis “host” or "symbiont" conventionally:

"host" indicates ones being larger and seeming to host the others; "symbiont" indicates the hosted ones.

Not to mention hosts exploited in parasitism, even mutualism often involves costs for the participants (Bronstein, 2001a). For example, legumes allocate 15-20 % of their photosynthetic products into root nodules for keeping rhizobia (Minchin & Pate, 1973), and other land plants pay 20% of the total carbon budget to mycorrhizal fungi (Johnson *et al.*, 1997). In this way, to benefit partners is costly for organisms, and thus, most of mutualisms involve costs as well as benefits (Bronstein, 2001a). Therefore, mutualism is sometimes considered as mutual exploitation where the benefit of exploiting partners exceeds the cost of being exploited by them (Antonovics *et al.*, 2015).

The fact that mutualism involves costs for participants raises evolutionary questions (Bronstein, 2001b; Wilkinson & Sherratt, 2001; Sachs & Simms, 2006; Akçay, 2015): why do they incur symbiotic costs to invest in their partners, why is mutual exploitation so mild that both of partners can enjoy symbiotic benefit, and why can mutualism be maintained without falling into parasitism? The participants who elicit an aid from their partner but never help it back in return can be evolutionarily successful. Such an individual, called “free-rider”, “cheater”, or “defector”, should enjoy an evolutionary advantage over a “cooperator” who does help its partner in return (Jones *et al.*, 2015). Note that although this distinction is not always rigorous, these terms such as “free-rider” are usually used for cheating individuals in mutualistic species compared with cooperators, whereas “parasite” is used for species devoting themselves to the exploitation of partners (Paracer & Ahmadjian, 2000; West *et al.*, 2007; Frederickson, 2013; Jones *et al.*, 2015).

The concept of free-riding is not merely hypothetical, but phenomena that considered as free-riding has actually been reported. For example, in the legume-rhizobium symbiosis, it is known that there are cheating strains of rhizobia that do not fix a sufficient amount of nitrogen for the host plants and can proliferate more rapidly than a nitrogen-fixing one by saving resources for the fixation (Kiers *et al.*, 2003; Kiers & Denison, 2008). Bumblebees sometimes rob nectar of flowers by making a hole in the bottom without pollinating (Maloof & Inouye, 2000). Even in mitochondria, a kind of "petit" mutants, which cannot work but proliferate faster than wild types, can dominate in a host cell (Chen & Clark-Walker, 1999). Thus, it is theoretically expected that cheaters will invade the population of cooperative symbionts and that they will ultimately break down the mutualism.

However, the prediction contradicts the facts that mutualism is ubiquitous and stable (Moran & Wernegreen, 2000; Wilkinson & Sherratt, 2001; Sachs & Simms, 2006; Frederickson, 2013). Many phylogenic studies have shown that mutualism has been stably maintained over an evolutionary time scale (Moran & Wernegreen, 2000; Sachs & Simms, 2006; Frederickson, 2013). In particular, even when mutualism broken down, it was usually followed by the end of symbiosis, namely the return to free-living, and falling into parasitism occurred rarely. In summary, mutualism is supposed to be vulnerable to the spread of free-riders and face the threat of falling into parasitism, but actually, it is maintained stably. This inconsistency indicates that there must be some mechanisms that discourage free-riding during costly mutualism (Bull & Rice, 1991; Sachs *et al.*, 2004; Lehmann & Keller, 2006; Nowak, 2006b; Akçay, 2015).

1.3. Mechanisms of evolutionary maintenance of mutualism

Despite the folklore that mutualism has been long neglected and unfocused on in favor of competition and prey-predator interactions in evolutionary ecology, this situation has changed dramatically in the last three decades along with the shift of the way biologists viewed the evolution of "cooperation", an interaction between individuals that benefits recipients but not necessarily the actor (Herre *et al.*, 1999; Akçay, 2015). Because mutualism can be considered as mutual cooperation between species, the evolution of mutualism also became one of the most important questions in evolutionary ecology, and huge theoretical and empirical literature has proposed the evolutionary mechanisms that maintain costly mutualism and prevent free-riding and exploitation (Foster & Wenseleers, 2006; Akçay, 2015).

Various mechanisms, which have been proposed, are based on "directed reciprocity", which is described by slogans: *you scratch my back, and I'll scratch yours*; *an eye for an eye, a tooth for a tooth* (Trivers, 1971; Axelrod & Hamilton, 1981; Bull & Rice, 1991; Sachs *et al.*, 2004). In directed reciprocation, an individual accepts a cost to benefit a specific partner, and the partner in turn compensates or reciprocates that benefit back to the benefiting individual. Under the situation, to invest in partners can be regarded as indirect investment to oneself and thus, advantageous for the actor. Therefore, various mechanisms for the maintenance of mutualism can be categorized depending on how directed reciprocity works, that is how the mechanism ensures that individual investing more receive more in return. In particular, depending on whether it works based on conditional responses by the partners or without such response, they are usually classified into "discrimination" and "partner fidelity feedback", as follows (Bull & Rice, 1991; Sachs *et al.*, 2004; Foster & Wenseleers, 2006; Frederickson, 2013; Jones *et al.*, 2015).

"Discrimination" is an active behavioral response to the quality or behavior of partners to reward cooperators but not free-riders or less-cooperative partners. By the discrimination, cooperators can be advantageous over free-riders because the former receives symbiotic benefit preferentially, and thus, discrimination can promote the evolution of cooperation by the partners. Because it is enough for evolutionary maintenance of mutualism that symbiotic benefits of cooperators are relatively higher than cheaters, discrimination can also include choosing only cooperators as a symbiotic partner, breaking off the symbiotic relationship with free-riders, and penalizing free-riders, for example. A well-known example is legumes that penalize non-cooperative rhizobia by halting oxygen and nutrient supply to them (Kiers *et al.*, 2003). Depending on the manner and species doing discrimination, these behaviors are called "sanction" (Denison, 2000; West *et al.*, 2002; Kiers *et al.*, 2003; Jandér & Herre, 2010), "partner choice" (Bull & Rice, 1991; Bever, 2002; Sachs *et al.*, 2004; Edwards *et al.*, 2006; Foster & Kokko, 2006), and "selective abortion" (Pellmyr & Huth, 1994; Goto *et al.*, 2010). In particular, the class of these behaviors is often called "partner choice" rather than "discrimination" (Bull & Rice, 1991; Sachs *et al.*, 2004; Frederickson, 2013). However, here I avoid using "partner choice" in such a broad sense, because it is also used in a narrow sense to indicate choosing symbiotic partners and thus it can lead to confusion.

"Partner fidelity feedback" is an automatic positive feedback between the fitness of participants involved in symbiosis (Bull & Rice, 1991; Sachs *et al.*, 2004; Foster & Wenseleers, 2006). The "automatic" means that the fitness interests of the participants can be aligned automatically through the structure of symbiotic association, such as the transmission mode and the duration of symbiotic association, without the

behavioral responses depending on partner's quality. Partner fidelity feedback can occur typically through “vertical transmission”, where a host transmits its symbionts to the offspring, because individuals can facilitate their own reproduction by increasing the fecundity of their partners (Fine, 1975; Yamamura, 1996; Frank, 1997; Sachs *et al.*, 2004; Foster & Wenseleers, 2006). Moreover, the feedback can occur within a generation; if individuals help their partners to survive, then they can increase their own benefits totally throughout the prolonged association with the partners. This type of feedback can play an essential role in the evolution of optimal virulence of parasites, where parasites face the tradeoff between the ability of spreading to new hosts and extending the lifetime of the current host (Frank, 1996b; Sachs *et al.*, 2004; Frederickson, 2013). In addition to vertical transmission and long lasting association, population viscosity can also induce the partner fidelity feedback (Doebeli & Knowlton, 1998; Yamamura *et al.*, 2004).

1.4. Evolution of the maintenance mechanisms of mutualism

Although it has been revealed theoretically how mutualism is maintained evolutionarily, the evolution of symbiotic relationship is still unclear because it remains to be solved how discrimination and partner fidelity feedback themselves evolve. As above, mutualism cannot evolve without these mechanisms. The evolution of these mechanisms is therefore an essential question to be investigated for understanding the evolution of symbiosis, in particular how symbiosis evolves toward not parasitism but mutualism.

Here, I theoretically explore the evolution of discrimination and partner fidelity feedback in detail. In chapter 2, I analyze the coevolutionary dynamics of

discrimination by hosts and cooperation by symbionts and show under what condition the mutualism maintained by discrimination can emerge and be maintained. In particular, I try to resolve the coevolutionary paradox of discrimination and cooperation, namely, domination by discriminating hosts depletes the variability in symbiont quality, which is usually considered as the source of selective advantage of discrimination, and thereby leads to the evolutionary loss of discrimination in hosts. In chapter 3, I examine when symbionts evolve to limit their division rate to synchronize with the host's cell division. Synchronized cell division is a driving force of partner fidelity feedback in mutualism between unicellular hosts and their endosymbionts, because it is a mechanism implementing vertical transmission in the mutualism and it leads to a permanent relationship between the hosts and symbionts. In chapter 4, I then discuss the evolutionary transition between mutualism maintained by discrimination and that by partner fidelity feedback based on the results of previous chapters. The discussion will provide an insight into the evolutionary emergence of organelles.

2. Evolutionary emergence and maintenance of horizontally transmitted mutualism

2.1. Introduction

Discrimination is one of the mechanisms for maintenance of mutualism and is to break off the relationship with free-riders and reward only cooperators (Bull & Rice, 1991; Noë & Hammerstein, 1995; Ferrière *et al.*, 2002; West *et al.*, 2002; Sachs *et al.*, 2004). A well-known example is legumes that penalize non-cooperative rhizobia by halting oxygen and nutrient supply to them (Kiers *et al.*, 2003). Such a mechanism is not limited to legumes but has been reported in many different mutualistic systems (reviewed by Frederickson (2013)). As legumes, plants hosting arbuscular mycorrhizal fungi also allocate more nutrients to the more beneficial fungi (Kiers *et al.*, 2011). Yuccas, figs, and *Glochidion* trees are known to abort their own seeds and fruits that contain too many eggs of their pollinator (yucca moths, fig wasps, and *Epicephala* moths, respectively), whereas they tolerate those which contain a few eggs (Pellmyr & Huth, 1994; Goto *et al.*, 2010; Jandér & Herre, 2010). Ant plants grow their 'domatia' that provide a habitat for their symbiotic ants, only if they are protected effectively by the ants (Edwards *et al.*, 2006). Leaf cutter ants preferentially cultivate cooperative and native strains of fungi (Mueller *et al.*, 2004; Mueller, 2012). Client fish in a cleaning mutualism stay with cooperative cleaners but leave cheaters which bite them (Bshary & Schäffer, 2002). These mechanisms have been called "sanction" (Denison, 2000; West *et al.*, 2002; Kiers *et al.*, 2003; Jandér & Herre, 2010), "partner choice" (Bull & Rice, 1991; Bever, 2002; Sachs *et al.*, 2004; Edwards *et al.*, 2006; Foster & Kokko, 2006), and "selective abortion" (Pellmyr & Huth, 1994; Goto *et al.*, 2010). As mentioned in

Introduction, I call these phenomena “discrimination”, regardless of the finer details (also see Steidinger & Bever (2014)).

Although discrimination promotes the evolution of cooperation, it has been argued that discrimination by hosts and cooperation by symbionts cannot be maintained through their coevolutionary dynamics if discrimination is costly (Foster & Kokko, 2006; McNamara *et al.*, 2008). The reason is paradoxical: once discrimination by hosts effectively removes free-riders from the symbiont population and cooperators become prevalent, a host can almost always meet cooperators and hence does no longer need to discriminate among symbionts. In such circumstance, the cost of maintaining discrimination would exceed its benefit, leading to evolutionary loss of discrimination in hosts. In other words, discrimination by hosts reduces variability in symbiont quality (level of cooperation by symbionts), only to undermine the selective advantage of discrimination (Foster & Kokko, 2006). Therefore, discrimination by hosts and the variability in partner quality cannot easily be maintained simultaneously, being considered as an evolutionary paradox (Foster & Kokko, 2006; Heath & Stinchcombe, 2014; Steidinger & Bever, 2014). In particular, the existence of the variability in partner quality is called “new paradox in mutualism” by Heath and Stinchcombe (2014), which is logically similar to the “lek paradox” of female choice and variability of male traits (Foster & Kokko, 2006). It has been proposed that sufficient supply of free-riding symbionts by exogenous mechanisms (i.e. mutation or immigration) is necessary to overcome the paradox, because it helps to maintain the benefit of discrimination and hence cooperation itself (Foster & Kokko, 2006; McNamara *et al.*, 2008). Others suggest that discrimination is not an evolutionary countermeasure against free-riding but merely a byproduct of the adaptation to environmental heterogeneity or defective

symbionts (Frederickson, 2013).

However, even though previous studies have emphasized the paradoxical feature of the evolution of costly discrimination and the existence of variability in symbiont quality (Foster & Kokko, 2006; Frederickson, 2013; Heath & Stinchcombe, 2014), it is theoretically still unclear how general the notion of the paradox is. It has been already proposed that, apart from constant supply by mutation or immigration, diversity in symbiont quality may be maintained by coevolutionary cycles in discrimination by hosts and cooperation by symbionts (Steidinger & Bever, 2014). Even more important is that different types of discrimination may allow the evolutionary maintenance of costly discrimination even without constant supply of variability in symbiont quality, which I study in this chapter. As introduced above, there is great diversity in the ways of host discrimination (Foster & Wenseleers, 2006; Frederickson, 2013). For example, yucca plants only abort seeds including eggs of rapacious pollinators and such abortion does not lead to new interactions, while abandoning a cheating cleaner fish provides an opportunity to find a new partner to the client fish. However, there has been little attempt to compare different types of discrimination and their effect on the coevolution of host discrimination and symbiont cooperation, and the relationship between the maintenance of discrimination and variability in symbiont quality.

An objective of my study is to reexamine the "paradoxical" coevolution of discrimination by hosts and cooperation by symbionts, by means of comparing two types, which I call "one-shot" and "resampling" types, of discrimination and analyzing the corresponding coevolutionary dynamics. As contrasted between yucca plants and client fish, host discrimination may be classified according to whether or not eviction of

free-riders is followed by the resampling of symbionts. In my one-shot type of discrimination, a host terminates the service to symbionts that are judged as free-riders, and does not resample new symbionts. In my resampling type of discrimination, the host terminates the service to symbionts as before, but then resamples new symbionts from the environment. I will show that the slight difference between the two ways of discrimination can lead to qualitative difference between their evolutionary outcomes: the costly discrimination of the one-shot type can maintain itself without any exogenous supply of free-riders; whereas the resampling discrimination requires sufficient supply of free-riders for being maintained. The latter result is consistent with the paradox shown by the previous studies; McNamara *et al.* (2008) assumed discrimination with partner resampling, and in Foster & Kokko (2006), discrimination is assumed as preferential provision of mutual aid to the more cooperative symbionts. I will then study the reason why these dichotomous results follow for each of different types of discrimination, and discuss whether the evolution of costly discrimination and cooperation is such paradoxical as has previously been emphasized (Foster & Kokko, 2006; Frederickson, 2013; Heath & Stinchcombe, 2014)

Another objective of my study is to find the condition for the emergence of costly discrimination by hosts from the population of non-discriminating hosts and non-cooperative symbionts. I will show that my “one-shot type” discrimination allows a discriminator to invade the population, which is impossible in the “resampling type” discrimination. I again theoretically highlight the reason leading to this dichotomy.

The third objective of this chapter is to study the effect of “assessment period” on the coevolutionary dynamics. In my model, an assessment period refers to an initial period of symbiosis during which a host evaluates the quality or the level of cooperation

of its partners; the host therefore cannot avoid exploitation by free-riding symbionts during this period. I expect that a longer assessment period may contribute to retaining free-riding symbionts, as this assessment period given by discriminators to free-riders would protect the free-riders even in the discriminator-only host population. Parallel discussions are found in the context of "lek paradox" (Kokko *et al.*, 2007) and "imperfect discrimination", where a host fails to discriminate free-riders with a small probability (Denison, 2000; Kiers & Denison, 2008; Friesen & Mathias, 2010). However, few studies on the evolution of discrimination mechanisms have focused on the effect of assessment period and imperfect discrimination.

Discrimination is often classified depending on the presence or absence of assessment period: if a host has to have time for assessment in discriminating symbionts, such discrimination type is called "sanction"; otherwise, it is called "partner choice" (Kiers & Denison, 2008; Frederickson, 2013). Thus, this classification of discrimination by hosts corresponds to the difference between evaluation mechanisms: sanction if it is based on the actual performance and partner choice if based on the signals reflecting the level of cooperation. Although this difference has been conceptually important in the context of the evolution of "honest signals", it is unclear whether the difference between sanction and partner choice affects the evolutionary dynamics of discrimination – the theoretical examination of which is the fourth and the last objective of the present chapter.

2.2. Model

I considered two species, a host and symbiont, which could benefit from cooperating with each other. I assumed discriminator and non-discriminator phenotypes in the host,

and cooperator and free-rider phenotypes in the symbiont. For simplicity, I assumed that both populations are infinitely large so that random genetic drift can be ignored, that both hosts and symbionts are haploid, and that both discrimination and cooperation traits are genetically determined. I then considered the dynamics of the frequency of discriminators in the host population and that of cooperators in the symbiont population.

Partnership formation: To model the process by which a host forms a symbiotic partnership with symbionts, I assumed that each host acquires a fixed number of symbionts randomly chosen from the symbiont population. The assumption of the fixed number of symbionts per host can be justified if the symbiont population is sufficiently larger than the host population. I also assumed that a host cannot distinguish symbiont phenotypes at the time of their acquisition (except for $\delta = 0$ as mentioned below).

Assessment period and eviction of free-riders: A non-discriminating host maintains the symbiotic relationship with whatever symbionts it acquires for an entire symbiotic period. Here, I rescaled time so that the total duration of symbiosis is normalized to one. On the other hand, a discriminating host spends time δ $(0 \leq \delta \leq 1)$ for assessing the quality of symbionts after their first acquisition (Fig. 2.1). During this assessment period, a discriminator cannot distinguish cooperators from free-riders. At the end of the assessment period, however, the discriminator can evict all the free-riders from the acquired symbionts, leaving only cooperators. Note that this discrimination is often called "partner choice" if $\delta = 0$, otherwise (i.e. if $\delta > 0$) it is often called "sanction" (Frederickson, 2013). my model with assessment period is mathematically equivalent to the model of imperfect discrimination, in which the assessment period δ is replaced by

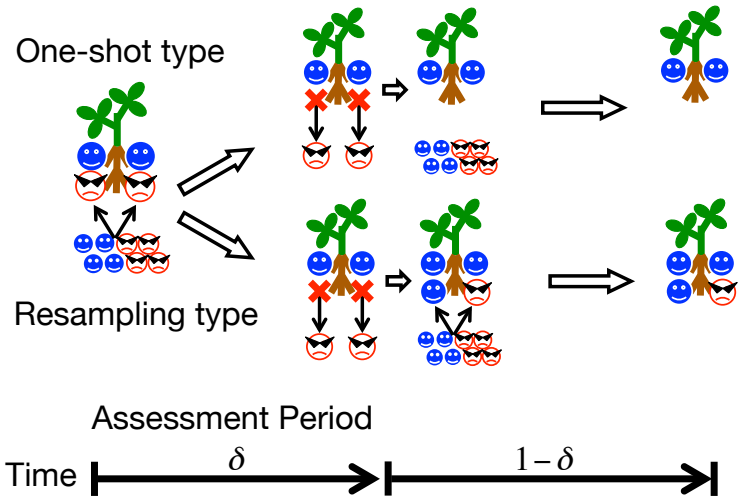

Figure 2.1. Schematic diagram of the models. Cartoons of a plant, a blue face, and a red face indicate a discriminator, a cooperator, and a free-rider, respectively. A discriminator discriminates free-riders from cooperators after an assessment period δ has elapsed from the initial acquisition. A resampling discriminator reacquires as many new symbionts as it evicted. On the other hand, a one-shot discriminator does not reacquire new symbionts.

the probability δ that a discriminator misjudges free-riders as cooperators (under this interpretation of δ, the execution cost parameter Δ_{E} defined later should be multiplied by $(1-\delta)$), though with repeated resampling discussed in Appendix A this interpretation of δ fails. my results derived in the assessment period model can therefore be applied to the model of imperfect discrimination.

Discrimination by host: As the discriminator's response after the eviction of free-riders, I considered two contrasting types, one-shot and resampling discrimination (Fig. 2.1): 1) In *one-shot* discrimination, a discriminating host lives only with the

	Initially sampled symbionts			Resampled symbionts*	
	Cooperator	Free-rider		Cooperator	Free-rider
	q	$1-q$		$(1-q)q$	$(1-q)^2$
Discriminator p	$B-C,\ b-c$	$-\delta C,\ \delta b$	The chance of resampling* $p(1-q)$	$(1-\delta)(B-C),\ (1-\delta)(b-c)$	$-(1-\delta)C,\ (1-\delta)b$
Non-discriminator $1-p$	$B-C,\ b-c$	$-C,\ b$		---	---

* By resampling discriminator only

Table 2.1. Host and symbiont payoffs from symbiotic interactions. The left and right entries separated by commas in each element of payoff matrix indicate the expected symbiotic payoff of a host and a symbiont, respectively. Symbols B and b correspond to the symbiotic benefit of hosts and symbionts, respectively. Similarly, symbols C and c correspond to the symbiotic cost of hosts and symbionts, respectively. These are expressed in units of per capita growth rate. The term δ indicates the length of assessment period (scaled by [0,1]). The symbols p and q represent frequency of discriminators in the host population and cooperators in the symbiont population, respectively.

cooperative symbionts left after eviction without attempting to reacquire further symbionts for the rest of its life that lasts for the time of length $1-\delta$; 2) in *resampling* discrimination, the host reacquires the same number of symbionts from the environment as the evicted free-riders at the time of discrimination. It then lives the rest of its life with a mixture of firstly acquired cooperators, resampled cooperators, and resampled free-riders. The host may iterate the acquiring-evicting process several times more during a finite period of symbiosis, but each time it needs another assessment period δ

to discriminate among resampled symbionts. When a host does not have enough time for the assessment after the last acquisition, it cannot discriminate against free-riders resampled at the acquisition. In the main text, because my analysis shows that the coevolutionary outcome is changed qualitatively depending on whether or not a host resamples new symbionts rather than how many times a host resamples, I assumed the simplest case in which discriminators can perform such discrimination only once in their symbiotic relationship with symbionts. In section 3.5 and Appendix A, I show the corresponding results when more than two discrimination events are allowed.

Payoffs with one-shot discrimination: A host is assumed to receive symbiotic benefit from cooperators but none from free-riders. The maximum benefit a host can receive per unit time if all symbionts are cooperators is denoted by $B\ (>0)$. The host is assumed to pay a symbiotic cost $C\ (>0)$ per unit time to help any type of symbiont. Let q and $1-q$ be the frequency of cooperators and free-riders in the symbiont population, respectively. The payoff of a discriminating host gained during assessment period is $\delta\{q(B-C)-(1-q)C\}$, as the initially acquired symbionts consist of the ratio $q:1-q$ of cooperators and free-riders; whereas, the payoff from post-assessment period is $(1-\delta)q(B-C)$, as only cooperators are left after eviction. Summing these up, I have the average payoff of a discriminator

$$\phi_{\mathrm{D}}^{0} = q(B-C)-(1-q)\delta C, \tag{2.1a}$$

(Table 1). The average payoff of a non-discriminator is $q(B-C)-(1-q)C$, or

$$\phi_{\mathrm{N}}^{0} = qB-C. \tag{2.1b}$$

A cooperative symbiont is assumed to receive a symbiotic benefit $b\ (>0)$ from the host per unit time and pay a symbiotic cost $c\ (>0)$ per unit time for helping

the host, whereas a free-riding symbiont is assumed to receive the same symbiotic benefit but do not pay any symbiotic cost in return. The average payoff of a cooperator is

$$\psi_{\mathrm{c}}^{\mathrm{O}} = b - c, \tag{2.2a}$$

because both discriminator and non-discriminator behave identically to a cooperator. Let p and $1-p$ be the frequency of discriminators and non-discriminators in the host population. The payoff of a free rider is

$$\psi_{\mathrm{f}}^{\mathrm{O}} = (1-p)b + p\delta b, \tag{2.2b}$$

because it can get the benefit from a discriminator only before it is evicted at the end of the assessment period (Table 2.1).

Payoffs with resampling discrimination: With resampling type discrimination, a discriminator reacquires the same number of symbionts as it evicted free-riders at time δ. The average payoffs of a resampling type discriminator and a non-discriminator are therefore

$$\phi_{\mathrm{D}}^{\mathrm{R}} = \phi_{\mathrm{D}}^{\mathrm{O}} + (1-q)(1-\delta)(qB - C), \tag{2.3a}$$

$$\phi_{\mathrm{N}}^{\mathrm{R}} = \phi_{\mathrm{N}}^{\mathrm{O}} = qB - C. \tag{2.3b}$$

The average payoff of a resampling discriminator is increased by $(1-q)(1-\delta)(qB - C)$ from that of a one-shot discriminator because it evicts $1-q$ of free-riders and reacquire the same number of symbionts and interact with them for the duration $1-\delta$, and the average payoff from resampled symbionts is the same as that of a non-discriminator, $qB - C$ (Table 2.1).

The average payoffs of a cooperator and a free-rider are

$$\psi_{\mathrm{c}}^{R} = \psi_{\mathrm{c}}^{\mathrm{O}} + p(1-q)(1-\delta)(b-c), \tag{2.4a}$$

$$\psi_{\mathrm{f}}^{R} = \psi_{\mathrm{f}}^{\mathrm{O}} + p(1-q)(1-\delta)b. \tag{2.4b}$$

The average payoff of a cooperator and that of a free-rider when discriminators are the resampling type are increased respectively by $p(1-q)(1-\delta)(b-c)$ and $p(1-q)(1-\delta)b$ compared with when discriminators are the one-shot type, because the fraction $p(1-q)$ of initially acquired symbionts are evicted by discriminators, and replaced by symbionts randomly resampled from the environment (Table 2.1). The resampled symbionts then interact with discriminators for the duration $1-\delta$.

Coevolutionary dynamics with one-shot discrimination: Combining the payoffs, Eq. (2.1) and Eq. (2.2), for host and symbiont phenotypes, with the cost of discrimination $\Delta = \Delta_{\mathrm{E}}(1-q) + \Delta_{\mathrm{M}}$, where the first term is execution cost, which I assume a discriminator incurs by discriminating free-riders, and the second term is the maintenance cost, which I assume a discriminator pays anytime, the changes in the frequency p of discriminator and the frequency q of cooperator can be described by the replicator-mutator equations (Nowak, 2006a), $dp/dt = \left(\phi_{\mathrm{D}}^{\mathrm{O}} - \phi_{\mathrm{N}}^{\mathrm{O}} - \Delta\right)p(1-p)$ and $dq/dt = \left(\psi_{\mathrm{c}}^{\mathrm{O}} - \psi_{\mathrm{f}}^{\mathrm{O}}\right)q(1-q) - \mu q$, or

$$\frac{dp}{dt} = \{(1-q)(1-\delta)C - \Delta_{\mathrm{E}}(1-q) - \Delta_{\mathrm{M}}\}p(1-p), \tag{2.5a}$$

$$\frac{dq}{dt} = \{p(1-\delta)b - c\}q(1-q) - \mu q, \tag{2.5b}$$

where μ is rate at which a free-rider is generated by mutation from a cooperator.

Coevolutionary dynamics with resampling discrimination: When the discrimination is resampling type, the payoffs for host and symbiont phenotypes are given by Eq. (2.3) and Eq. (2.4) with the discrimination cost of Δ, which yields $dp/dt = \left(\phi_{\mathrm{D}}^{\mathrm{R}} - \phi_{\mathrm{N}}^{\mathrm{R}} - \Delta\right)p(1-p)$ and $dq/dt = \left(\psi_{\mathrm{c}}^{\mathrm{R}} - \psi_{\mathrm{f}}^{\mathrm{R}}\right)q(1-q) - \mu q$, or

$$\frac{dp}{dt} = \{q(1-q)(1-\delta)B - \Delta_{\mathrm{E}}(1-q) - \Delta_{\mathrm{M}}\}p(1-p), \tag{2.6a}$$

$$\frac{dq}{dt} = \{p(1-\delta)b - c - p(1-q)(1-\delta)c\}q(1-q) - \mu q. \tag{2.6b}$$

The outcome of coevolutionary dynamics for discrimination by host and cooperation by symbiont are then studied by analyzing the system (2.5) for the one-shot discrimination case and (2.6) for the resampling discrimination case.

2.3. Results

2.3.1. Coevolution with one-shot discrimination

The coevolutionary dynamics of the frequencies of one-shot discriminators, p, and cooperators, q, given by Eq. (2.5), revealed four qualitatively different outcomes depending mainly on the two key parameters, the mutation rate μ and the duration of assessment period δ (Fig. 2.2; see Appendix B for the full analysis).

Firstly, if assessment period δ is larger than the critical value $\hat{\delta}$ (defined by Eq. (B2) in Appendix B), the host population cannot maintain one-shot discriminators and the symbiont population cannot maintain cooperators (Fig. 2.2*A*), leading to the breakup of mutualism. In contrast, if the assessment period for discrimination is

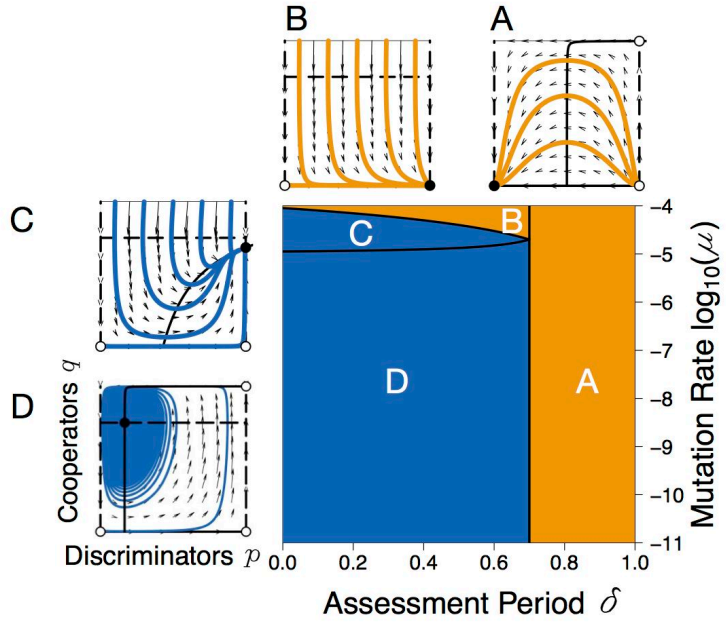

Figure 2.2. The effect of assessment period δ and mutation rate μ on the coevolutionary dynamics of one-shot discriminators and cooperators. (Main panel) Both discriminators and cooperators are maintained at a stable equilibrium when a pair of the parameters (δ, μ) is in the blue region. In contrast, both go extinct in the orange region. Boundaries of *A*– (*B*–*D*), *B*–*C*, and *C*–*D* correspond to $\delta = \hat{\delta}$, $\mu = \hat{\mu}_1$, and $\mu = \hat{\mu}_2$, respectively. (Sub panel *A*–*D*) Each panel shows typical coevolutionary trajectories in each parameter region. The horizontal and vertical axes indicate frequencies of discriminators and cooperators, respectively, ranging from 0 to 1. Colored, solid, and dashed lines respectively indicate trajectories of coevolutionary dynamics, nullclines of discriminator dynamics, and nullclines of cooperator dynamics. Black and white circles indicate stable and unstable equilibria. The parameter values $(\delta, \log_{10}(\mu))$ in Figures *A*–*D* are $(0.8, -8)$, $(0.4, -4.2)$, $(0.4, -4.8)$, and $(0.4, -8)$, respectively. The other values are $C = 5.0 \times 10^{-5}$, $\Delta_{\mathrm{E}} = 1.0 \times 10^{-5}$, $\Delta_{\mathrm{M}} = 5.0 \times 10^{-6}$, $b = 1.0 \times 10^{-4}$, and $c = 1.0 \times 10^{-5}$.

sufficiently short, $\delta < \hat{\delta}$, costly discrimination can stably be maintained regardless of the rate of mutation that generates free-riders.

Secondly, if the assessment period is shorter than the threshold ($\delta < \hat{\delta}$) and the mutation rate μ is larger than the first, higher threshold $\hat{\mu}_1$(Eq. (B2) in Appendix B), the host and symbiont population become monomorphic, consisting only of discriminators and of free-riders (Fig. 2.2*B*). The reason why cooperators cannot invade in the symbiont population, despite the fact that they would avoid eviction by hosts, is that the effect of the mutational loss of cooperators overcomes their selective advantage -- this corresponds to an error catastrophe in symbiont quasi-species (Eigen & Schuster, 1977). Hence, in this parameter region, hosts always release all the associated symbionts after the assessment period; mutualistic relationship is not established.

Thirdly, if the mutation rate is in between the first and the second thresholds, $\hat{\mu}_2 < \mu < \hat{\mu}_1$, the host population is still fixed to discriminators, but the symbiont population becomes polymorphic with cooperators and free-riders (Fig. 2.2*C*). Since the mutation rate becomes small, the state of the symbiont population changes from the error catastrophe to the mutation-selection balance. The equilibrium frequencies of discriminators and cooperators are determined by this mutation-selection balance.

Lastly, if the mutation rate is smaller than the second, lower threshold $\hat{\mu}_2$, both host and symbiont populations become polymorphic (Fig. 2.2*D*). It should be noted that discriminators and cooperative symbionts are stably maintained in polymorphic populations however low is the mutation rate. In this region where the mutation rate is sufficiently small, the equilibrium frequencies of discriminators and cooperators are determined by frequency-dependent selection, rather than mutation-selection balance. In contrast to the resampling type model described below,

the frequency-dependent selection in the one-shot discrimination model does not destabilize the polymorphic steady state in the host and symbiont populations.

Note that the monomorphic population consisting only of free-riders in symbiont and non-discriminator in host is always unstable as long as the assessment period is short sufficiently ($\delta < \hat{\delta}$; bottom left corner points in Fig. 2.2B-D). This means that, unlike the case of resampling discrimination described below, one-shot discriminators can invade the non-mutualistic population, even if the standing variation for symbiont quality is absent.

In summary, discriminators can be maintained without exogenous supply of free-riders even if the discrimination is costly (Fig. 2.2). Moreover, cooperators are also maintained unless the mutation rate μ is so high as to outweigh natural selection ($\hat{\mu}_1 < \mu$; Fig. 2.2*B*). In addition, the assessment period δ must not be too long to prevent the extinction of discriminators and cooperators (Fig. 2.2*B* to Fig. 2.2*D*).

2.3.2. Coevolution with resampling discrimination

When the discrimination is resampling type, the coevolutionary outcome of the dynamics (Eq. 6) again depends mainly on the assessment period and mutation rate, but there are 6, rather than 4, qualitatively different outcomes of the coevolutionary dynamics (Fig. 2.3; see Appendix C for. mathematical analysis of this system). The results are summarized as follows.

If the assessment period δ is too long ($\delta > \hat{\delta}^{\mathrm{R}}$; see Appendix C for the definition of $\hat{\delta}^{\mathrm{R}}$), neither discriminator nor cooperator can be maintained regardless of the value of the mutation rate μ (Fig. 2.3*A*). As free-riders go to fixation in this region,

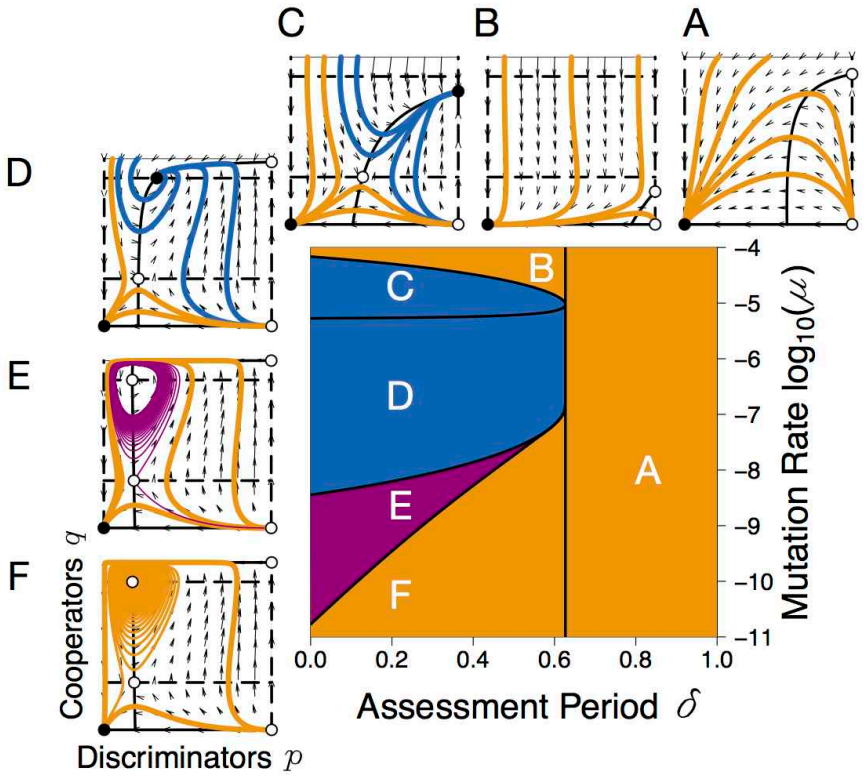

Figure 2.3. The effect of assessment period δ and mutation rate μ on the coevolutionary dynamics of rejection discriminators and cooperators. (Main panel) The blue and orange regions are the same as in Fig. 2. Both discriminators and cooperators are maintained in a stable limit cycle when a pair of parameters(δ, μ) is in the purple region. Boundaries of *A*– (*B*–*F*), *B*–*C*, *C*–*D*, *D*–*E*, and *E*–*F* correspond to $\delta = \hat{\delta}^{\mathrm{R}}$, $\mu = \hat{\mu}_1^{\mathrm{R}}$, $\mu = \hat{\mu}_2^{\mathrm{R}}$, $\mu = \hat{\mu}_3^{\mathrm{R}}$, and $\mu = \hat{\mu}_4^{\mathrm{R}}$, respectively. (Sub panel *A*–*F*) Each axis and each line is the same as in Fig. 2. Parameter values $\left(\delta, \log_{10}(\mu)\right)$ of Panels *A*–*F* are $(0.8, -6)$, $(0.4, -4.44)$, $(0.4, -5)$, $(0.4, -6)$, $(0.4, -8)$, and $(0.4, -10)$, respectively. The other values are $B = 1.0 \times 10^{-4}$, $C = 5.0 \times 10^{-5}$, $\Delta_{\mathrm{E}} = 1.0 \times 10^{-5}$, $\Delta_{\mathrm{M}} = 5.0 \times 10^{-6}$, $b = 1.0 \times 10^{-4}$, and $c = 1.0 \times 10^{-5}$.

the hosts have no choice but to obtain the free-riders because discrimination is not effective enough to justify its costs.

Given that the assessment period δ is sufficiently short ($\delta < \hat{\delta}^{\mathrm{R}}$), the evolutionary outcomes changes with mutation rate as follows: both discriminators and cooperators go extinct at a very high mutation rate (Fig. 2.3*B*). As the mutation rate goes below the first threshold, coevolutionary bistability arises: non-discriminator/free-rider equilibrium is still locally stable, but there exists another locally stable equilibrium in which the host population becomes fixed to discriminators, and the symbiont population is polymorphic with cooperators and free-riders (Fig. 2.3*C*). As the mutation rate becomes smaller than the second threshold, both the host and symbiont populations become polymorphic in the latter equilibrium (Fig. 2.3*D*). At a mutation rate even smaller than the third threshold, discriminators and cooperators can be maintained with a stable coevolutionary oscillation (Fig. 2.3 *E*). Finally, if the mutation rate becomes smaller than the fourth threshold, both discriminators and cooperators go extinct again (Fig. 2.3*F*). Note that the magnitude of mutation rate required for the maintenance is always positive unless the maintenance cost of discrimination Δ_{M} is zero (Eq. C4 in Appendix C). Therefore, costly discrimination and cooperation require moderately high rate of mutation for being maintained stably.

The monomorphic population consisting only of free-riders in symbiont and non-discriminator in host is always locally stable regardless of the values of the focal parameters, μ or δ (bottom left corner points in Fig. 2.3A-F). Therefore, there is bistability between non-mutualistic and mutualistic states in the parameter regions C-E (Fig. 2.3). This means that, unlike the case of one-shot discrimination, neither discriminators nor cooperators can invade the population when they are rare if the

discrimination is resampling type.

In summary, the mutation rate μ must be moderately high to stably maintain discriminators and cooperators in the population (Fig. 2.3*C* to Fig. 2.3*E*). This result suggests that variability must be introduced into symbiont quality via mutation to maintain costly discrimination and the mutualistic relationship. Thus, the evolution of the resampling discrimination corresponds to the paradoxical result of the previous studies (Foster & Kokko, 2006; McNamara *et al.*, 2008). In particular, my model qualitatively reproduces the simulation results of Foster & Kokko (2006); i.e., a transition between different evolutionary outcomes along with the change in mutation rate (Foster & Kokko (2006); Fig. 2.3, p.5). In addition, as with the one-shot type, the assessment period δ must not be too long to prevent the extinction of discriminators and cooperators (Fig. 2.3*C* to Fig. 2.3*F*).

2.3.3. Emergence of discrimination

In contrast to the resampling discriminators, the one-shot discriminators can invade the free-rider/non-discriminator population, even if standing variation in symbiont quality is absent. In my model, a direct advantage of a resampling discriminator over a non-discriminator is the additional benefit earned from the cooperators it adopts after the assessment period. It is represented as $q(1-q)(1-\delta)B$ in Eq. (2.6a), which is proportional to the *symbionts' diversity* $2q(1-q)$ (measured by the probability that two individuals taken at random from the symbiont population represent different types). On the other hand, a direct advantage of a one-shot discriminator over a non-discriminator is to avoid the further cost of continuing to help free-riding symbionts.

It is represented as $(1-q)(1-\delta)C$ (Eq. (2.5a)), which is proportional to the frequency of free-riders $1-q$, rather than the symbionts' diversity. Thus, if free-riders dominate the symbiont population ($q \approx 0$), the resampling discriminators obtain the minimum benefit, but the one-shot discriminators get the largest one. Thus, in contrast to the resampling type, one-shot discrimination can easily emerge from a non-mutualistic population without standing variation in symbiont quality.

2.3.4. Maintenance of discrimination

The one-shot discrimination can be maintained however low is the mutation rate, while the resampling discrimination requires moderately high rate of mutation for being maintained. The latter result is consistent with the previous paradoxical results shown by other authors. In my model, the maintenance of the resampling type discrimination is challenged by positive feedback in cooperator frequency dynamics due to the inability of discriminating resampled free-riders. Since resampled symbionts are not discriminated, resampled cooperators suffer disadvantage over resampled free-riders. Thus, the more frequent are cooperators, the less is the number of resampled symbionts after eviction, the less is the selective disadvantage for resampled cooperators, and cooperators become more favorable for the natural selection. This positive feedback in the dynamics of cooperator frequency breaks down the evolutionary maintenance of the resampling discrimination (mathematically speaking, the dominant eigenvalue of the Jacobian matrix governing the stability of the polymorphic equilibrium with resampling discriminators and non-discriminators becomes positive by adding the term $p(1-q)(1-\delta)c$ that corresponds to the disadvantage of resampled cooperators seen in Eq.

(2.6b) to Eq. (2.5b) of the one-shot type). In contrast to these complications in the resampling type, the one-shot type induces no such positive feedback in cooperator frequency dynamics and hence induces no destabilizing effect.

2.3.5. Coevolutionary dynamics with repeated resampling discrimination

In my model, resampling discriminators are assumed to perform discrimination (eviction and resampling) only once in their symbiotic relationship with symbionts. Here I briefly discuss how the results change by relaxing this assumption. In the model of repeated resampling, although the fact remains that a host cannot evict free-riders after the last acquisition of symbionts, I found that the repetition has a stabilizing effect; If the number of repetitions is large enough, stable maintenance of discrimination is possible without exogenous supply of free-riders (see Appendix A).

In contrast to the maintenance, the emergence of discrimination is still difficult even with a large number of repetitions, because such discriminators cannot invade the population if standing variation in symbiont quality is absent (Appendix A).

2.4. Discussion

In this study, I tried to resolve the paradox of discrimination by hosts and cooperation by symbionts, namely, domination by discriminators depletes the variability in symbiont quality and thereby drains the benefit of discrimination, by comparing two different types of discrimination as examples, the one-shot and resampling discrimination (Fig. 2.1). My study has shown that this apparently minor difference in discrimination types leads to qualitatively different evolutionary outcomes. In particular, my study has

provided three novel findings regarding the evolution of discrimination mechanisms.

First, although it has been usually considered that the benefit of discriminators is derived from the variability of partner quality (West *et al.*, 2002; Foster & Kokko, 2006; McNamara *et al.*, 2008), I have shown that the benefit of a certain type of discriminators (e.g. one-shot discrimination) is proportional to the frequency of free-riding, rather than the phenotypic diversity in the degree of cooperation as in the resampling discrimination case. Indeed, the selective advantage for resampling discriminators, which is proportional to the symbionts' diversity $2q(1-q)$, is maximized when cooperators and free-riders segregate in one-to-one ratio but vanishes when the genetic diversity is lost in symbionts. As a result, one-shot type discriminators can invade the free-rider/non-discriminator population, even if standing variation in symbiont quality is absent.

Second, my one-shot discriminators can also be maintained without exogenous supply of free-riders and hence is free from the paradox of discrimination. Unlike resampling type discrimination, one-shot discrimination does not induce the positive feedback in cooperator dynamics that destabilizes coevolutionary dynamics, and hence discriminators can be maintained without exogenous stabilizing forces such as mutation changing cooperators to free-riders. In addition, since repetition of resampling discrimination has a stabilizing effect, repeated-resampling discriminators can also be maintained stably without exogenous supply of free-riders (see Appendix A). Therefore, my result indicates that the paradox is not a common feature of evolution of discrimination but a problem specific to a certain model.

Third, a longer assessment period or a larger probability of a discriminator failing to discriminate among symbionts discourages maintenance of discrimination. I

expected that such effects may contribute to retaining free-riding symbionts and then help to maintain variability in symbiont quality and maintain discrimination, because it could help free-riders to escape from discrimination and makes the spread of discriminators difficult. However, at least in my model, imperfectness of discrimination can only weaken the strength of selection and therefore cannot contribute to the maintenance of variability in symbiont quality and that of discrimination. Moreover, the evolution of discrimination by hosts does not sensitively depend on the presence or absence of an assessment period in my model (compare the results for small positive δ and those for $\delta = 0$ in Fig. 2.2 and 3), i.e., the difference is not significant between sanction (positive δ) and partner choice ($\delta = 0$).

2.4.1. Biological interpretation

As I showed, the variability in partner quality can be maintained through frequency-dependent selection depending on the manner of discrimination. This result indicates that the maintenance of the variability in partner quality (in particular, the existence of low quality symbionts) can be achieved more easily than previously thought (Frederickson, 2013; Heath & Stinchcombe, 2014; Jones *et al.*, 2015). Therefore, even if discrimination seems to erode the variability in partner quality at first glance, the maintenance of the variability is actually not so paradoxical.

It is difficult to determine whether the maintenance of an observed discrimination actually depends on exogenous supply of variability, because the detailed manner of the discrimination is still unclear. However, I believe that my model can provide insight into several observations listed below. First, my one-shot discrimination

model can be applied to some of selective abortion systems (Pellmyr & Huth, 1994; Goto *et al.*, 2010; Jandér & Herre, 2010), because host plants abort fruits including rapacious pollinators only and such abortion is not followed by new interactions. If these mechanisms actually correspond to one-shot type, exogenous supply is not important to maintain discrimination. Although my model assumed that a free-rider can increase its fitness thanks to the symbiotic interaction during the assessment period, several discrimination mechanisms such as selective abortion by yucca are lethal for symbionts. However, even if free-riders cannot benefit from the symbiotic interaction sufficiently during the assessment period (e.g. not only being evicted but suffering additional penalty from the host), my results do not change qualitatively, although the parameter region in which both discriminators and cooperators are maintained becomes broader. Second, in contrast, my resampling discrimination model can be applied to partner choice by client fish (Bshary & Schäffer, 2002), because abandoning a cheating cleaner fish provides an opportunity to find a new partner for a client fish. This system seems to be similar to my resampling model and the theoretical model of McNamara *et al.* (2008). If these mechanisms actually correspond to resampling type, they may be maintained stably by exogenous supply of free-riders and/or repetition of discrimination. Finally, sanction by legumes against rhizobia reported by Kiers *et al.* (2003) seems to be a one-shot type if sanction does not lead to new nodulation, because legumes just halt oxygen and nutrient supply to free-riding rhizobia. On the other hand, if a legume reallocates the remaining nutrient, which was supposed to be consumed by free-riders if it did not penalize, to newly formed nodules, such sanction will correspond to resampling type rather than one-shot type above mentioned. Note that a discrimination mechanism is classified into the resampling type when eviction leads to new interaction.

Even if a host acquires new symbionts after discrimination, the discrimination is not classified into the resampling type when acquisition is induced not by eviction but seasonality or growth of hosts. Because several species of hosts can always resample new symbionts from their environment potentially, as legumes and host plants of mycorrhizal fungi do, there is a possibility that their discrimination corresponds to resampling type.

2.4.2. Limitations of my study and future directions

I proposed a simple mathematical model to focus on the relationships between the effect of recurrent supply of free-riders and discrimination type. However, this approach has several potential limitations.

Firstly, I have focused only on the resampling and one-shot discrimination among potentially diverse array of discrimination types. However, I think that my discussion can provide insight into the evolutionary dynamics of other kinds of discrimination. In my model, although spread of both one-shot and resampling discriminators reduces their several selective advantages indirectly, whether or not exogenous supply of free-riders is necessary to explain their maintenance differ from each other. The evolutionary feedback in which discriminators ultimately reduce their own fitness via the loss of diversity in symbiont quality, has been considered as the cause of the paradoxical results (Foster & Kokko, 2006; Frederickson, 2013; Heath & Stinchcombe, 2014), but my results indicate that other effects such as those discussed below are rather the direct cause of the paradoxical results. For example, McNamara *et al.* (2008) assumed a process corresponding to partner resampling after discrimination

in their one species model. In their model, if an individual evicts its partner, both individuals return to the pool of unpaired individuals, from which new pairs are randomly formed. Because an interaction is broken down by not only eviction but also the death of a partner, high death rate abates bias of eviction toward free-riders and disadvantages a cooperator over a free-rider. This effect probably corresponds to the destabilizing effect, which is derived from the inability of discriminating resampled free-riders, in the maintenance of resampling discrimination in my model. Moreover, resampling is not assumed in the model of Foster & Kokko (2006), but positive feedback is explicitly assumed as "partner fidelity feedback" in their model; if a focal individual increases its investment to its symbiotic partner, the symbiotic benefit that the focal individual receives in return increases through better growth and longer survival of the symbiotic partner by that additional investment. I believe that the partner fidelity feedback on the fitness of symbionts makes the evolution of discrimination paradoxical. On the other hand, the discrimination mechanism of Steidinger & Bever (2014) is maintained without the recurrent mutation generating free-riders. Similarly to my one-shot discrimination model, they do not assume resampling or other positive feedback. In fact, it is mathematically included in my one-shot model (their model is mathematically equivalent to my one-shot model when I set the mutation rate exactly equal to zero).

Secondly, my models are based on a simple genetic framework such as a one-locus two-allele model. Thus, strictly speaking, it is unclear whether my results can be directly applicable to a more complicated model treating quantitative traits such as some previous studies (Foster & Kokko, 2006; McNamara *et al.*, 2008). In spite of the difference in the framework between these models and mine, however, I believe that the

comparison of the results is still possible by paying attention to some of the key factors that affect the evolution of discrimination, e.g., whether the benefit depends mainly on the variability in symbiont quality (as in my resampling type model) or on the frequency of free-riders (as in one-shot type model), and whether the model induces positive feedback in evolutionary dynamics of cooperation in symbionts (as in my resampling model) or includes any built-in feedback like partner fidelity feedback, as I have discussed.

Thirdly, I assumed that discrimination functions as relentless sanction, by which a host completely evicts free-riders from its symbiotic relationship. Other milder forms of discriminations are also possible such as slightly reducing resource supply for free-riders or rewarding cooperators. I believe, however, that my model can also be applied to those cases by changing the values or interpretation of the parameters and yield similar results because these changes do not affect the structure of frequency dependent selection.

Finally, I did not consider the competition between the two discrimination types, because my purpose is not to reveal the evolution of the resampling or one-shot discrimination itself but to show that discriminators can be maintained without exogenous supply depending on the assumptions. However, the competition between several types of discrimination is also interesting. I have already shown in the present chapter that the one-shot type can spread over the non-discriminator population under much broader conditions than can the resampling type, but I also expect that the one-shot type may be vulnerable to invasion by the resampling type. One of the reasons for the latter is that the coevolutionary equilibrium of the one-shot type is a polymorphic population with cooperators and free-riders, which I can show that the

resampling type invades. The coevolutionary dynamics of the two different types of discrimination in hosts and cooperation by symbionts are complex and should be considered in a future study.

Discrimination mechanisms and partner fidelity feedback have been proposed as major evolutionary forces maintaining interspecific cooperation (Bull & Rice, 1991; Sachs *et al.*, 2004; Foster & Wenseleers, 2006). Moreover, it has been considered that the former is important in horizontally transmitted mutualism while the latter is thought to be essential in vertically transmitted mutualism (Sachs *et al.*, 2004). It has been suggested that a high mutation rate in symbionts is needed for the evolution of discrimination mechanisms (West *et al.*, 2002; Foster & Kokko, 2006; McNamara *et al.*, 2008) but this discourages the evolution of interspecific cooperation in vertically transmitted mutualism, because high genetic variability facilitates competition among symbionts within a host due to reduced relatedness (Frank, 1994, 1996a). Therefore, the origin of the transition from horizontally to vertically transmitted mutualism is considered to be difficult to explain. In contrast, my one-shot model potentially connects these two modes of mutualism because this discrimination mechanism does not require a high mutation rate. However, as mentioned above, the one-shot type may be vulnerable to the invasion by the resampling type. Thus, the relationship between discrimination mechanisms and competition between the two discrimination types is important to understand the transition from horizontally to vertically transmitted mutualism.

2.5. Appendix

2.5.1. Mathematical analysis of iterated resampling discrimination

Model

In the following analysis, I extend my resampling model to allow repeated discriminations by hosts. Unlike my original resampling model in the main body, once repeated evicting–resampling processes are allowed, the length of an assessment period, δ, can no longer be reinterpreted as the probability of recognition error, in which a host misconstrue a free-rider as a cooperator. Hereafter, therefore, I restrict the meaning of the parameter δ to the length of a single assessment period only.

Payoffs: Suppose that a discriminator iterates discrimination processes (eviction and resampling) n times, where $n\delta$ should be lower than one because the total assessment period $n\delta$ cannot exceed the total symbiotic period, which is normalized to one (see the section "Assessment period and eviction of free-riders" in the main text). In each round of discrimination, the fraction $1-q$ of free-riders are evicted from previously sampled symbionts, where q is frequency of cooperators in the population. Therefore, the fraction of free-riders evicted in the i-th discrimination is $(1-q)^i$ of all symbionts in a discriminating host. Hence, the fraction of cooperators resampled just after the i-th discrimination is $(1-q)^i q$, with which the discriminator maintains the symbiotic relationship for the rest of its symbiotic period $1-i\delta$. Therefore, the total benefit which a discriminating host acquires from symbionts is $\sum_{i=0}^{n}(1-q)^i\, q(1-i\delta)B$. Whereas, the discriminating host pays symbiotic cost C to any symbiont anytime in symbiotic period. Therefore, the average payoff of a discriminator is

$$\phi_{\mathrm{D}}^{n} = \sum_{i=0}^{n} (1-q)^{i} q(1-i\delta)B - C, \tag{A1}$$

where the term for $i = 0$ in the right hand side is the contribution from symbionts acquired before the first discrimination. The average payoff of a non-discriminator is the same as in the main body: $\phi_{\mathrm{N}}^{n} = qB - C$. Moreover, a discriminator incurs the costs of discrimination $\Delta = \sum_{i=1}^{n} (1-q)^{i} \Delta_{\mathrm{E}} + \Delta_{\mathrm{M}}$, where the first term is the execution cost and the second one is the maintenance cost.

As with the payoff of a discriminating host, the average payoffs of a cooperating and a free-riding symbiont are

$$\begin{aligned} \psi_{\mathrm{c}}^{n} &= (1-p)(b-c) + p \sum_{i=0}^{n} (1-q)^{i}(1-i\delta)(b-c), \\ \psi_{\mathrm{f}}^{n} &= (1-p)b + p \left\{ \sum_{i=0}^{n-1} (1-q)^{i} \delta b + (1-q)^{n}(1-n\delta)b \right\}, \end{aligned} \tag{A2}$$

where p is the frequency of discriminators in host and the first terms are derived from the association with non-discriminators and the second ones are from the association with discriminators. Note that for free-riders resampled after the last discrimination, the time for symbiotic interaction with a discriminator is $1 - n\delta$, rather than δ.

Coevolutionary dynamics: The changes in the frequency p of discriminator and the frequency q of cooperator can be described by replicator equations $dp/dt = (\phi_{\mathrm{D}}^{n} - \phi_{\mathrm{N}}^{n} - \Delta)p(1-p)$ and $dq/dt = (\psi_{\mathrm{c}}^{n} - \psi_{\mathrm{f}}^{n})q(1-q)$, or

$$\frac{dp}{dt} = \left[q(1-q)B \sum_{i=0}^{n-1} (1-q)^{i}\{1-(i+1)\delta\} - \Delta_{\mathrm{E}}(1-q) \sum_{i=0}^{n-1} (1-q)^{i} - \Delta_{\mathrm{M}} \right] p(1-p),$$

$$\frac{dq}{dt} = \left[pb \sum_{i=0}^{n-1} (1-q)^{i}\{1-(i+1)\delta\} - c - p(1-q)c \sum_{i=0}^{n-1} (1-q)^{i}\{1-(i+1)\delta\} \right] q(1-q),$$

where, unlike the main text, I ignore the mutation for the sake of simplicity. A little algebra shows that they become

$$\begin{aligned}\frac{dp}{dt} &= [\alpha_n(q)q(1-q)\{1-\delta_n(q)\}B - \alpha_n(q)\Delta_{\mathrm{E}}(1-q) - \Delta_{\mathrm{M}}]p(1-p),\\ \frac{dq}{dt} &= [\alpha_n(q)p\{1-\delta_n(q)\}b - c - \alpha_n(q)p(1-q)\{1-\delta_n(q)\}c]q(1-q),\end{aligned} \tag{A3}$$

where

$$\begin{aligned}\alpha_n(q) &= \sum_{i=0}^{n-1}(1-q)^i = \frac{1-(1-q)^n}{q},\\ \delta_n(q) &= \delta\frac{\sum_{i=0}^{n-1}(i+1)(1-q)^i}{\alpha_n(q)} = \delta\left\{1 - \frac{n(1-q)^n}{1-(1-q)^n} + \frac{1-q}{q}\right\}.\end{aligned} \tag{A4}$$

This transformation will be helpful to compare this model with the original model (Eq. 6).

Results

There are four trivial equilibria (p,q) in Eq. A3: $(0,0)$, $(0,1)$, $(1,0)$, and $(1,1)$. Only the first one, $(0,0)$, is stable, and the others are unstable, regardless of the values of the parameters. Therefore, neither discriminators nor cooperators can invade the non-mutualistic population consisting only of non-discriminating host and free-riding symbionts.

In addition to the trivial equilibria, there are at most two internal equilibria: $(P^n(\hat{q}_+^n), \hat{q}_+^n)$ and $(P^n(\hat{q}_-^n), \hat{q}_-^n)$, where

$$P^n(q) = \frac{c}{\alpha_n(q)\{1-\delta_n(q)\}\{b-(1-q)c\}}, \tag{A5}$$

and $\hat{q}_+^n$ and $\hat{q}_-^n$ are the roots of $f_n(q) \equiv \phi_{\mathrm{D}}^n - \phi_{\mathrm{N}}^n - \Delta = 0$ (i.e. $dp/dt = 0$ under $p \neq 0,1$; let $\hat{q}_+^n$ and $\hat{q}_-^n$ be $\hat{q}_+^n > \hat{q}_-^n$). It can be proved below that the number of internal equilibria in the interval $0 < q < 1$ is either 0 or 2. First, by substituting $q =$

$1-s$, where s denotes the frequency of free-riders, the polynomial $f_n(q)$ is rewritten as $f_n(q) = F_n(s)$ where

$$F_n(s) = s(1-s)B\sum_{i=0}^{n-1} s^i\{1-(i+1)\delta\} - \Delta_{\mathrm{E}} s\sum_{i=0}^{n-1} s^i - \Delta_{\mathrm{M}}. \tag{A6}$$

Since $n\delta < 1$, $F_n(s)$ is always negative when $s \geq 1$. Therefore, if $F_n(s)$ has positive roots, they must be in the interval $(0,1)$. Second, a little algebra shows that the polynomial $F_n(s)$ becomes

$$-(1-n\delta)Bs^{n+1} - \sum_{i=2}^{n}\{\delta B + \Delta_{\mathrm{E}}\}s^i + \{(1-\delta)B - \Delta_{\mathrm{E}}\}s - \Delta_{\mathrm{M}}. \tag{A7}$$

This is a polynomial ordered by descending exponent from s^{n+1} to the constant $s^0 = 1$. If the coefficient $(1-\delta)B - \Delta_{\mathrm{E}}$ of s is positive, the polynomial $F_n(s)$ has two sign changes between consecutive nonzero-coefficients (i.e., between the terms of s^2 and s and between the terms s and s^0). By Descartes' rule of signs, having two sign changes indicates that the number of positive roots of the equation $F_n(s) = 0$ is either two or zero. As a result, the equation $F_n(s) = 0$, i.e. $f_n(q) = 0$, has at most two roots in the interval $(0,1)$. Therefore, there are two internal equilibria, $(P^n(\hat{q}_+^n), \hat{q}_+^n)$ and $(P^n(\hat{q}_-^n), \hat{q}_-^n)$, under appropriate sets of parameters (Δ_{E}, Δ_{M}, and δ must be small enough for $F_n(s)$ to be positive in a part of the interval $(0,1)$, and c must also be small enough for the term $b-(1-q)c$ to be positive). Moreover, because $f_n(0) = -n\Delta_{\mathrm{E}} - \Delta_{\mathrm{M}}$ and $f_n(1) = -\Delta_{\mathrm{M}}$ are negative, it follows that the derivatives of $f_n(q)$ evaluated at two internal equilibria if they exist, $\partial f_n(\hat{q}_-^n)/\partial q$ and $\partial f_n(\hat{q}_+^n)/\partial q$, are positive and negative, respectively.

The equilibrium $(P^n(\hat{q}_+^n), \hat{q}_+^n)$ can be stable if n is large and c is small enough. This can be shown by evaluating the signs of the determinant and the trace of

the Jacobi matrix at the equilibrium in the following way, because it is known in general that an equilibrium in a two-dimensional system is stable if and only if its determinant and trace of the Jacobian matrix are positive and negative, respectively. Let us write $\psi_{\mathrm{c}}^{n} - \psi_{\mathrm{f}}^{n}$ as $g_n(p,q)$, because it is a polynomial of p and q. The Jacobi matrix of the dynamics

$$\frac{dp}{dt} = f_n(q)p(1-p),$$
$$\frac{dq}{dt} = g_n(p,q)q(1-q)$$

evaluated at the equilibrium is:

$$J = \begin{pmatrix} 0 & \dfrac{\partial f_n(\hat{q}_+^n)}{\partial q} P^n(\hat{q}_+^n)\left(1 - P^n(\hat{q}_+^n)\right) \\ \dfrac{\partial g_n(P^n(\hat{q}_+^n), \hat{q}_+^n)}{\partial p} \hat{q}_+^n(1-\hat{q}_+^n) & \dfrac{\partial g_n(P^n(\hat{q}_+^n), \hat{q}_+^n)}{\partial q} \hat{q}_+^n(1-\hat{q}_+^n) \end{pmatrix}. \quad \text{(A8)}$$

Because the denominator of $P^n(\hat{q}_+^n)$ is equal to $\partial g_n(P^n(\hat{q}_+^n), \hat{q}_+^n)/\partial p$, $\partial g_n(P^n(\hat{q}_+^n), \hat{q}_+^n)/\partial p$ is positive when the equilibrium exists. Therefore, the determinant of the Jacobi matrix, which is given by $-J_{12}J_{21}$, is positive (note that $\partial f_n(\hat{q}_+^n)/\partial q$ is negative as I mentioned above). Moreover, because the trace of the Jacobi matrix, which is equal to J_{22}, is negative if $\partial g_n(P^n(\hat{q}_+^n), \hat{q}_+^n)/\partial q$ is negative. In summary, the equilibrium $(P^n(\hat{q}_+^n), \hat{q}_+^n)$ is stable if $\partial g_n(P^n(\hat{q}_+^n), \hat{q}_+^n)/\partial q$ is negative, where

$$\frac{\partial g_n(P^n(\hat{q}_+^n), \hat{q}_+^n)}{\partial q} = -P^n(\hat{q}_+^n) \sum_{i=1}^{n-1} \{(1-i\delta)(b-c) - \delta b\} i (1-\hat{q}_+^n)^{i-1} + (1-n\delta)cP^n(\hat{q}_+^n)n(1-\hat{q}_+^n)^{n-1}. \quad \text{(A9)}$$

This can be negative if n is large and c is small enough, because it converges to $-(b-c)/q^2$ as $n \to \infty$ (note that since the total assessment period $n\delta$ cannot exceed the total symbiotic period, δ has to become infinitesimal simultaneously). The

first term in the right hand side represents an evolutionary advantage of cooperators resampled at the i-th acquisition ($i = 1, \cdots, n-1$), and the second term is the disadvantage of a cooperator resampled at the last acquisition. Note that a cooperator acquired at the last round is disadvantageous over a free-rider resampled at the same time, because the discriminator cannot discriminate between them anymore (see the section 3.4 in the main text).

In contrast to the equilibrium $(P^n(\hat{q}_+^n), \hat{q}_+^n)$, the other internal equilibrium, $(P^n(\hat{q}_-^n), \hat{q}_-^n)$, is unstable because $\partial f_n(\hat{q}_-^n)/\partial q$ is positive and therefore the determinant is negative.

In summary, repeated-resampling discriminators can stably be maintained without any exogenous supply of free-riders under appropriate conditions. In contrast to the maintenance, the emergence of the discrimination is still difficult because such discriminators cannot invade the population if standing variation in symbiont quality is absent.

2.5.2. Mathematical analysis of one-shot type discrimination model

In the following analysis I will pay attention to the positions of the two nullclines (namely, their zero growth isoclines) of the evolutionary dynamics (Eq. (2.5)). An intersection of these nullclines, if it exists, is an equilibrium of the dynamics, and I will study its local stability.

The nullclines for the frequency p of discriminators in equation (Eq. (2.5)) are three lines: p=0, p=1, and q=$\hat{q}$ (Fig. 2.3*A*–*D*), where

$$\hat{q} = 1 - \frac{\Delta_{\mathrm{M}}}{(1-\delta)C - \Delta_{\mathrm{E}}}. \tag{B1}$$

If the frequency of cooperator, q, is less than the threshold, $\hat{q}$, the benefit of discrimination exceeds its cost, $\Delta_{\mathrm{E}}(1-q)-\Delta_{\mathrm{M}}$, and thus discriminators increase in the host population (q=$\hat{q}$ is the dashed horizon in Fig. 2.2*B*–*D*). On the other hand, the nullclines for the frequency q of cooperators in equation (Eq. (2.5)) are $q=0$ and $p=P(q)$ (solid black lines in Fig. 2.2*A*–*D*), where

$$P(q)=\frac{c}{(1-\delta)b}+\frac{\mu}{(1-q)(1-\delta)b}.$$

If the frequency of discriminators exceeds the threshold, $P(q)$, cooperators increase in the symbiont population ($p=P(q)$ is the solid black curve in Fig. 2.2*A*, 2*C*, and 2*D*).

There are therefore 4 possible equilibria: $(p,q)=(0,0)$, $(1,0)$, $(P(\hat{q}),\hat{q})$, and $(1,\hat{Q})$, where

$$\hat{Q}=1-\frac{\mu}{(1-\delta)b-c}.$$

The third one corresponds to the intersection of the nullclines, $p=P(q)$ and q=$\hat{q}$. Similarly, the last one is the intersection of the nullclines, $p=P(q)$ and p=1.

By analyzing local stabilities of these equilibria, I see that there are 4 cases that yield qualitative different outcomes of the evolutionary dynamics (Eq. (2.5)), which can be separated by the following critical values in the parameter space of δ (assessment period) and μ (mutation rate):

$$\begin{aligned}\hat{\delta}&=1-\frac{\Delta_{\mathrm{E}}+\Delta_{\mathrm{M}}}{C},\\ \hat{\mu}_1&=(1-\delta)b-c,\\ \hat{\mu}_2&=(1-\hat{q})\{(1-\delta)b-c\}.\end{aligned} \tag{B2}$$

Firstly, if the assessment period δ is larger than the critical value $\hat{\delta}$, the equilibrium $(P(\hat{q}),\hat{q})$ is out of the range of frequencies because the threshold $\hat{q}$ is below 0. Among the rest of three equilibria, only $(0,0)$ is stable (in fact, globally stable) (Fig.

2.2*A*). Thus, the host population cannot sustain discriminators, and the symbiont population cannot maintain cooperators, either.

Secondly, if the assessment period δ is shorter than $\hat{\delta}$ and the mutation rate μ is larger than $\hat{\mu}_1$ defined above, the nullcline $q = \hat{q}$ exists in the phase space but the nullcline curve $p = P(q)$ does not (Fig. 2.2*B*). Therefore, there are only two equilibria: $(0,0)$, which is unstable and $(1,0)$, and which is stable (in fact, globally stable). At the equilibrium (1,0), all hosts are discriminators and all symbionts are free-riders. Hence, hosts always release all their symbionts to the environment after an assessment period, which is far from stable symbiosis.

Thirdly, if the mutation rate, μ, passes through the critical value $\hat{\mu}_1$ from above but stays above the lower critical value ($\hat{\mu}_2 < \mu < \hat{\mu}_1$), while the assessment period being kept less than the threshold ($\delta < \hat{\delta}$), the nullcline curve, $p = P(q)$ rises up into the feasible frequency ranges and hence the equilibrium $(1, \hat{Q})$ appears from the line $q = 0$ (transition from Fig. 2.2*B* to Fig. 2.2*C*). Transcritical bifurcation occurs at the boundary; the equilibrium (1,0) loses its stability and the equilibrium $(1, \hat{Q})$ becomes stable. The equilibrium $(0,0)$ is unstable as before. Therefore, $(1, \hat{Q})$ is the unique stable equilibrium (Fig. 2.2*C*). At the equilibrium $(1, \hat{Q})$, the host population is fixed to discriminators, and the symbiont population is polymorphic with cooperators and free-riders.

Lastly, if the mutation rate μ becomes less than the second critical value, $\hat{\mu}_2$ (i.e. $0 < \mu < \hat{\mu}_2$ and $\delta < \hat{\delta}$), the stable equilibrium $(1, \hat{Q})$ crosses the nullcline $q = \hat{q}$, and the equilibrium $(P(\hat{q}), \hat{q})$ comes into the feasible frequency range $0 \leq p, q \leq 1$ after it collides with $(1, \hat{Q})$ (transition from Fig. 2.2*C* to Fig. 2.2*D*). When this occurs,

the equilibrium $(1, \hat{Q})$ loses its stability and the new equilibrium $(P(\hat{q}), \hat{q})$ becomes stable (transcritical bifurcation). The equilibria $(0,0)$ and $(1,0)$ remain unstable as before. Therefore, $(P(\hat{q}), \hat{q})$ is the unique stable equilibrium (Fig. 2.2*D*). At the equilibrium $(P(\hat{q}), \hat{q})$, the host and symbiont population are polymorphic with discriminators and non-discriminators, and with cooperators and free-riders, respectively.

Moreover, if the mutation rate is zero ($\mu = 0$ and $\delta < \hat{\delta}$), the equilibrium $(P(\hat{q}), \hat{q})$ is neutrally stable. It can be proved by defining a conservation quantity $V(p, q)$ can be defined as follows:

$$V(p,q) = \ln p^{-c}(1-p)^{-\{(1-\delta)b-c\}} - \ln q^{\{(1-\delta)C-\Delta_{\mathrm{E}}-\Delta_{\mathrm{M}}\}}(1-q)^{-\Delta_{\mathrm{M}}}.$$

This is actually conserved on a coevolutionary trajectory ($\dot{V}(p,q) = 0$) as below:

$$\begin{aligned}\dot{V}(p,q) &= \frac{\partial V}{\partial p}\dot{p} + \frac{\partial V}{\partial q}\dot{q} \\ &= \frac{(1-\delta)bp - c}{p(1-p)}[\{(1-\delta)C - \Delta_{\mathrm{E}}\}(1-q) - \Delta_{\mathrm{M}}]p(1-p) \\ &\quad - \frac{\{(1-\delta)C - \Delta_{\mathrm{E}}\}(1-q) - \Delta_{\mathrm{M}}}{q(1-q)}\{(1-\delta)bp - c\}q(1-q) \\ &= 0.\end{aligned}$$

2.5.3 Mathematical analysis of resampling type discrimination model

The nullclines for the frequency of discriminators in equation (Eq. (2.6)) are p=0, p=1, q=$\hat{q}_{+}^{\mathrm{R}}$, and q=$\hat{q}_{-}^{\mathrm{R}}$ (Fig. 2.3*A* to Fig. 2.3*F*), where

$$\begin{aligned}\hat{q}_{+}^{\mathrm{R}} &= \frac{1}{2}\left\{1 + \frac{\Delta_{\mathrm{E}}}{(1-\delta)B} + \sqrt{(1 - \frac{\Delta_{\mathrm{E}}}{(1-\delta)B})^2 - \frac{4\Delta_{\mathrm{M}}}{(1-\delta)B}}\right\}, \\ \hat{q}_{-}^{\mathrm{R}} &= \frac{1}{2}\left\{1 + \frac{\Delta_{\mathrm{E}}}{(1-\delta)B} - \sqrt{\left(1 - \frac{\Delta_{\mathrm{E}}}{(1-\delta)B}\right)^2 - \frac{4\Delta_{\mathrm{M}}}{(1-\delta)B}}\right\}.\end{aligned} \tag{C1}$$

Only when the frequency of cooperators, q, falls between the critical frequencies, $\hat{q}_+^{\mathrm{R}}$ and $\hat{q}_-^{\mathrm{R}}$, the benefit of discrimination exceeds its cost, $\Delta_{\mathrm{E}}(1-q)-\Delta_{\mathrm{M}}$, and thus discriminators increase in the host population ($q=\hat{q}_+^{\mathrm{R}}$ and $q=\hat{q}_-^{\mathrm{R}}$ are the upper and the lower dashed horizons respectively in Fig. 2.3*B* to Fig. 2.3*F*), otherwise discriminators decrease in frequency. The nullclines for the frequency of cooperators in equation (Eq. (2.6)) are $q=0$ and $p=P^{\mathrm{R}}(q)$ (Fig. 2.3*A* to Fig. 2.3*F*), where

$$P^{\mathrm{R}}(q)=\frac{(1-q)c+\mu}{(1-q)(1-\delta)\{b-(1-q)c\}}. \tag{C2}$$

If the frequency of discriminators p exceeds the critical frequency, $P^{\mathrm{R}}(q)$, (the solid black curve in Fig. 2.3*A* to Fig. 2.3*F*) cooperators increase in the symbiont population, otherwise it decreases.

There are at most 6 more equilibria: $(p,q)=(0,0)$, $(1,0)$, $(P^{\mathrm{R}}(\hat{q}_+^{\mathrm{R}}),\hat{q}_+^{\mathrm{R}})$, $(P^{\mathrm{R}}(\hat{q}_-^{\mathrm{R}}),\hat{q}_-^{\mathrm{R}})$, $(1,\hat{Q}_+^{\mathrm{R}})$, and $(1,\hat{Q}_-^{\mathrm{R}})$, where

$$\begin{aligned}\hat{Q}_+^{\mathrm{R}}&=1-\frac{1}{2}\left\{\frac{(1-\delta)b-c}{(1-\delta)c}-\sqrt{\left(\frac{(1-\delta)b-c}{(1-\delta)c}\right)^2-\frac{4\mu}{(1-\delta)c}}\right\},\\ \hat{Q}_-^{\mathrm{R}}&=1-\frac{1}{2}\left\{\frac{(1-\delta)b-c}{(1-\delta)c}+\sqrt{\left(\frac{(1-\delta)b-c}{(1-\delta)c}\right)^2-\frac{4\mu}{(1-\delta)c}}\right\}.\end{aligned} \tag{C3}$$

The last two correspond with the intersection of p=1 and $p=P^{\mathrm{R}}(q)$ (the point $(1,\hat{Q}_-^{\mathrm{R}})$ is not in Fig. 2.3 under the condition of the figure). Since $(P^{\mathrm{R}}(\hat{q}_+^{\mathrm{R}}),\hat{q}_+^{\mathrm{R}})$ and $(1,\hat{Q}_+^{\mathrm{R}})$ are the only equilibria of which stability depends on parameter values, I will focus on them mainly. The equilibria $(1,0)$, $(P^{\mathrm{R}}(\hat{q}_-^{\mathrm{R}}),\hat{q}_-^{\mathrm{R}})$, and $(1,\hat{Q}_-^{\mathrm{R}})$ are always unstable regardless of parameter values. In contrast, the trivial equilibrium $(0,0)$ is always stable.

By analyzing local stabilities of these equilibria, I see that there are 6 cases

that yield qualitatively different outcomes of the evolutionary dynamics (Eq. (2.6)), which can be separated by the following critical values in the parameter space of δ (assessment period) and μ (mutation rate):

$$\hat{\delta}^{\mathrm{R}} = \min\left(1 - \frac{\Delta_{\mathrm{E}} + 2\left(\Delta_{\mathrm{M}} + \sqrt{(\Delta_{\mathrm{E}} + \Delta_{\mathrm{M}})\Delta_{\mathrm{M}}}\right)}{B}, 1 - \frac{c}{b}\left(1 + 2\frac{\mu + \sqrt{(b+\mu)\mu}}{b}\right)\right),$$

$$\begin{aligned} \hat{\mu}_1^{\mathrm{R}} &= (1-\hat{q}_-^{\mathrm{R}})\{(1-\delta)b - c - (1-\hat{q}_-^{\mathrm{R}})(1-\delta)c\}, \\ \hat{\mu}_2^{\mathrm{R}} &= (1-\hat{q}_+^{\mathrm{R}})\{(1-\delta)b - c - (1-\hat{q}_+^{\mathrm{R}})(1-\delta)c\}, \\ \hat{\mu}_3^{\mathrm{R}} &= \frac{(1-\hat{q}_+^{\mathrm{R}})^2 c^2}{b - 2(1-\hat{q}_+^{\mathrm{R}})c}, \\ \hat{\mu}_4^{\mathrm{R}} &, \end{aligned} \tag{C4}$$

where $\hat{\mu}_4^{\mathrm{R}}$ is not obtained analytically but is obtained by numerical simulations (Fig. 2.3). The inequality $\hat{\mu}_1^{\mathrm{R}} > \hat{\mu}_2^{\mathrm{R}} > \hat{\mu}_3^{\mathrm{R}} > \hat{\mu}_4^{\mathrm{R}} > 0$ is usually satisfied under the condition $\delta < \hat{\delta}^{\mathrm{R}}$.

(i) When the assessment period δ is too long ($\delta > \hat{\delta}^{\mathrm{R}}$), either the set of nullclines $q = \hat{q}_+^{\mathrm{R}}$ and $q = \hat{q}_-^{\mathrm{R}}$ or the nullcline $p = P^{\mathrm{R}}(q)$ does not exist (Fig. 2.3*A* shows the case in which the nullclines $q = \hat{q}_+^{\mathrm{R}}$ and $q = \hat{q}_-^{\mathrm{R}}$ are absent). Under this condition, the points $(P^{\mathrm{R}}(\hat{q}_+^{\mathrm{R}}), \hat{q}_+^{\mathrm{R}})$ and $(P^{\mathrm{R}}(\hat{q}_-^{\mathrm{R}}), \hat{q}_-^{\mathrm{R}})$ are outside the phase space. Moreover, the point $\left(1, \hat{Q}_+^{\mathrm{R}}\right)$ is also absent, or it is unstable even if it exists. Only equilibrium $(0,0)$ is stable, and thus discriminators and cooperators are never maintained.

(ii) When the assessment period δ is sufficiently short ($\delta < \hat{\delta}^{\mathrm{R}}$) but when the mutation rate μ is too high ($\mu > \hat{\mu}_1^{\mathrm{R}}$), the nullcline $p = P^{\mathrm{R}}(q)$ is below the nullcline $q = \hat{q}_-^{\mathrm{R}}$ (Fig. 2.3*B*). The values $P^{\mathrm{R}}(\hat{q}_+^{\mathrm{R}})$ and $P^{\mathrm{R}}(\hat{q}_-^{\mathrm{R}})$ exceed one, and thus the equilibria $(P^{\mathrm{R}}(\hat{q}_+^{\mathrm{R}}), \hat{q}_+^{\mathrm{R}})$ and $(P^{\mathrm{R}}(\hat{q}_-^{\mathrm{R}}), \hat{q}_-^{\mathrm{R}})$ still do not exist. Moreover, the point $\left(1, \hat{Q}_+^{\mathrm{R}}\right)$ is still unstable. Under this condition, discriminators and cooperators are never

maintained as in the previous condition (i).

(iii) When the mutation rate goes below the first critical value (i.e. $\hat{\mu}_2^{\mathrm{R}} < \mu < \hat{\mu}_1^{\mathrm{R}}$ and $0 < \delta < \hat{\delta}^{\mathrm{R}}$), the nullcline $p = P^{\mathrm{R}}(q)$ intersects with the nullcline $q = \hat{q}_-^{\mathrm{R}}$ (transition from Fig. 2.3*B* to Fig. 2.3*C*), and the new equilibrium $(P^{\mathrm{R}}(\hat{q}_-^{\mathrm{R}}), \hat{q}_-^{\mathrm{R}})$ emerges. It exchanges the stability with the equilibrium $(1, \hat{Q}_+^{\mathrm{R}})$ at $\mu = \hat{\mu}_1^{\mathrm{R}}$ (transcritical bifurcation). Therefore, $(P^{\mathrm{R}}(\hat{q}_-^{\mathrm{R}}), \hat{q}_-^{\mathrm{R}})$ is unstable, and $(1, \hat{Q}_+^{\mathrm{R}})$ is stable. There are two possibilities for the evolutionary outcome (Fig. 2.3*C*). The first one is $(1, \hat{Q}_+^{\mathrm{R}})$, where discriminators are fixed, and where cooperators coexist with free-riders. The second one is $(0,0)$, where both discriminators and cooperators are extinct.

(iv) Similarly, when the mutation rate μ becomes below the second critical value (i.e. $\hat{\mu}_3^{\mathrm{R}} < \mu < \hat{\mu}_2^{\mathrm{R}}$ and $0 < \delta < \hat{\delta}^{\mathrm{R}}$), the nullcline $p = P^{\mathrm{R}}(q)$ intersects with the nullcline $q = \hat{q}_+^{\mathrm{R}}$ (transition from Fig. 2.2*C* to Fig. 2.2*D*), and the new equilibrium $(P^{\mathrm{R}}(\hat{q}_+^{\mathrm{R}}), \hat{q}_+^{\mathrm{R}})$ emerges. It exchanges the stability with the equilibrium $(1, \hat{Q}_+^{\mathrm{R}})$ at $\mu = \hat{\mu}_2^{\mathrm{R}}$ (transcritical bifurcation). Therefore, $(P^{\mathrm{R}}(\hat{q}_+^{\mathrm{R}}), \hat{q}_+^{\mathrm{R}})$ is stable, and $(1, \hat{Q}_+^{\mathrm{R}})$ becomes unstable. Therefore, there are two evolutionary outcomes depending on the initial condition: discriminators and non-discriminators coexist in the host population and cooperators and free-riders coexist in the symbiont population at $(P^{\mathrm{R}}(\hat{q}_+^{\mathrm{R}}), \hat{q}_+^{\mathrm{R}})$, or both discriminators and cooperators go extinct at $(0,0)$ (Fig. 2.3*D*).

(v) When the mutation rate μ becomes lower than the third threshold (i.e. $\hat{\mu}_4^{\mathrm{R}} < \mu < \hat{\mu}_3^{\mathrm{R}}$ and $0 < \delta < \hat{\delta}^{\mathrm{R}}$), the point where the frequency of discriminators p is the minimum value on the nullcline $p = P^{\mathrm{R}}(q)$ rises above the stable equilibrium $(P^{\mathrm{R}}(\hat{q}_+^{\mathrm{R}}), \hat{q}_+^{\mathrm{R}})$. At the same time, it becomes unstable, and a stable limit cycle surrounding it arises (Hopf bifurcation: transition from Fig. 2.3*D* to Fig. 2.3*E*).

Therefore, under this condition, discriminators and cooperators are maintained with coevolutionary oscillation, or they go extinct at $(0,0)$ (Fig. 2.3*E*).

(vi) As the mutation rate, μ, further decreases, the amplitude of the stable limit cycle becomes larger. When the mutation rate μ becomes lower than a certain value (i.e. $\mu < \hat{\mu}_4^{\mathrm{R}}$ and $0 < \delta < \hat{\delta}^{\mathrm{R}}$), the stable limit cycle collides with the equilibrium $(P^{\mathrm{R}}(\hat{q}_-^{\mathrm{R}}), \hat{q}_-^{\mathrm{R}})$ and then disappears (homoclinic bifurcation). Therefore, there are no stable equilibria or limit cycles except for the equilibrium $(0,0)$. In this case, discriminators and cooperators are not maintained anymore (Fig. 2.3*F*)

3. Evolutionary emergence and maintenance of vertical transmission in mutualism

3.1. Introduction

Lineages under symbiosis sometimes evolve to merge into a single inseparable unit of organism, such as plastids and their hosts (Margulis 1981; Cavalier-Smith 2013; Mereschkowsky 1905 translated by Martin & Kowallik 1999). The evolutionary process driving symbionts to organelles, which is called "symbiogenesis", is a core of the "major transition" (Maynard Smith and Szathmáry 1995) from prokaryotes to eukaryotes and is the major challenge in evolutionary biology to understand the origin of eukaryote and organelle evolution.

One of the key steps of symbiogenesis is thought to be the evolution of synchronized cell division between endosymbionts and the unicellular host (Margulis, 1981; Inouye & Okamoto, 2005; Rodriguez-Ezpeleta & Philippe, 2006; Keeling & Archibald, 2008). If endosymbionts proliferate faster than their host, they accumulate and impose a heavy burden on the host that may induce its death or burst. In contrast, if their proliferation is slower than their host's, they are left behind by host's proliferation and eventually are lost from the host. Therefore, synchronized cell division is necessary for the permanent relationship between endosymbionts and their host, and the evolution towards reproductive synchrony is necessary at some point of symbiogenesis. In fact, the synchronized cell divisions by various mechanisms have been reported in mutualisms between unicellular hosts and their endosymbionts (e.g. Kadono *et al*. 2004; Takahashi *et al*. 2007; Motta *et al*. 2010).

Because endosymbionts would be able to divide potentially faster than the

host, symbionts must limit their cell division in order to synchronize with their host's. If hosts are exposed to the risk of excessive accumulation of symbionts in their cell, limiting symbionts' cell division is potentially advantageous for hosts. On the other hand, it is apparently paradoxical that symbionts would self-limit their own cell division rate because it is the principal component of their fitness. Therefore, an evolutionary question that arises here is whether symbionts can evolve to restrain their own cell division. Even if a host controls the cell division of its symbionts as in conventional wisdom (Keeling & Archibald, 2008; Motta *et al.*, 2010; Nowack & Melkonian, 2010; Dean *et al.*, 2016; Lowe *et al.*, 2016), it is still to be asked whether symbionts would accept such an enforced limitation of their cell division by the host.

A possible reason why symbionts would benefit from limiting their own division is that accelerated cell division will damage the host and miss their opportunity to be transmitted to the daughter host cells (vertical transmission). However, it is by no means obvious whether this explanation would actually work, because the accelerated cell division will also help symbionts to be released from the host via its death, providing them a chance to spread to new hosts (horizontal transmission). Therefore, dividing faster than the host decreases symbionts' chance for vertical transmission on the one hand, but increases their chance for horizontal transmission on the other. Symbionts therefore face the tradeoff between vertical and horizontal transmission through the evolution of division rate. It is well known that pathogens that face similar tradeoff through the evolution of virulence cannot usually lead to obligatory vertically transmitted pathogens, because it such pathogens should go extinct as, without horizontal transmission, the infected hosts are out-competed by uninfected hosts (Lipsitch *et al.*, 1996). By contrast, mutualistic symbionts may prevent such extinction

by benefiting their host that harbors them, and they may evolve to limit their own cell division for facilitating vertical transmission.

Here, I theoretically examine when symbionts evolve to limit their division to synchronize with the host's cell division. I constructed a mathematical model that is inspired by the mutualism between unicellular hosts and their symbionts, such as host ciliate and symbiotic algae. In my model of coevolution between hosts and symbionts, I assume that the symbionts can control their division rates in the host, while the hosts can control the mortality rate of the associated symbionts. The tradeoff between vertical and horizontal transmissions in my model is naturally implemented through my explicit modeling of symbiont dynamics within a host cell. The interaction between hosts and symbionts in my model is either mutualistic or parasitic; namely, although the symbionts always benefit their host, the host can incur more harm than it receives the benefit if their division rate is too high. My theory can cast light on resolving dichotomous views of symbiogenesis: whether it is an outcome of evolutionary conflict ending up with symbionts' reconciling themselves to limit proliferation or an evolutionary equilibrium where both host and symbionts benefit from synchronized cell division.

3.2. Model

To examine the evolution of vertical transmission through self-limited cell division of symbionts, I assume that the cell division rate of symbionts in a host cell is controlled by themselves and hence is regarded as an evolutionary trait of *symbionts*. I also assume that the death rate of symbionts in a host cell is controlled by hosts, and hence is regarded as an evolutionary trait of *hosts*. Here I do not specify how hosts control the

death of symbionts, but such control is possible in various ways. For example, hosts can increase the mortality of symbionts via intracellular digestion and resistance against pathogenic symbionts. They can also reduce it via the supply of nutrients to symbionts and physical protection of them from their enemies. Hereafter I call the trait of hosts, which controls the death rate of their symbionts, "host generosity"; a generous host tries to keep symbionts within its cell longer and more securely, while an ungenerous host tries to kill its symbionts rapidly.

In order to investigate the evolution of these phenotypes, the self-limited cell division of symbionts and the host generosity to symbionts, I employed the evolutionary invasion analysis of adaptive dynamics (Geritz *et al.*, 1998), where evolutionary dynamics is considered as a sequential trait substitution by invasion of mutants in the equilibrium population of residents. Therefore, I first constructed the model of population dynamics of hosts and symbionts, and then examined the invasibility of a rare mutant into the equilibrium population of the resident.

3.2.1. Population dynamics of symbionts and hosts

I consider population dynamics of two species, a unicellular host and its intracellular symbiont (Fig. 3.1). For modeling symbiont dynamics within a host cell explicitly, I classify the host cells according to the number of symbionts residing its cell. Here, for simplicity, I assume that a host can keep only up to two symbionts (see Discussion for the results when a host can harbor more than two symbionts) and thus keep track of time evolution of the density H_0 of free-living hosts, the densities H_i of hosts that harbor i symbionts ($i = 1,2$). I also keep track of the density S of free-living

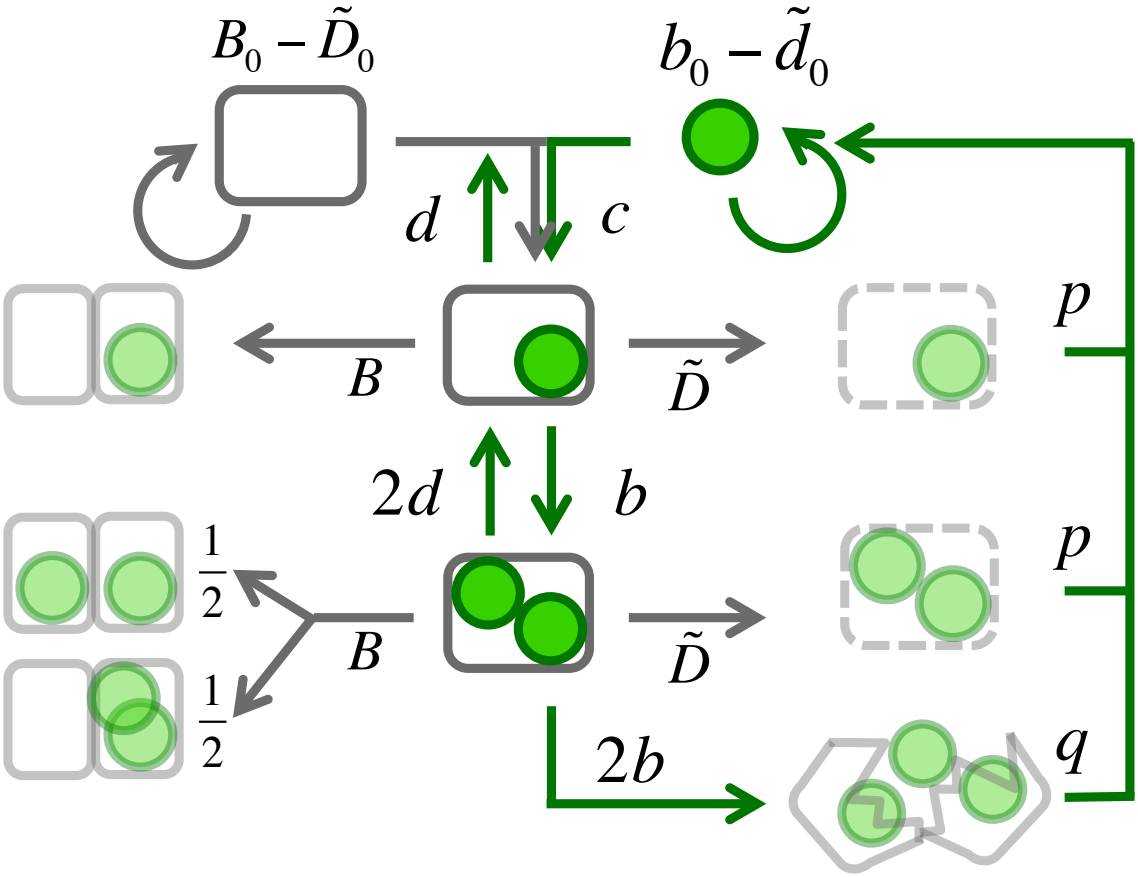

Figure 3.1. Schematic diagram of my model. Gray squares and green circles indicate host and symbiont cells, respectively. Transparent symbols represent the transient states during the division (left side) and death (right side) of symbiotic hosts, and they are not independent variables of the dynamics. Gray and green arrows are respectively the transition induced by a host and symbiont. The definitions of parameters are described in the main text and Table 3.1.

symbionts. These densities change with time as follows (Fig. 3.1). (i) *Birth and death of free-living hosts.* A free-living host proliferates at a rate B_0 per unit time (hereafter, all the rates are those per unit time) and die at a mortality rate $\widetilde{D}_0 = D_0 \sum_{i=0}^{2} H_i$, where I assumed that the mortality is proportional to the total host density with a proportionality constant D_0. (ii) *Birth and death of free-living symbionts*. A free-living symbiont proliferates and dies at a rate b_0 and $\tilde{d}_0 = d_0 S$, where I also assumed that the mortality is proportional to the density of free living symbionts, S, with a proportionality constant d_0. Since symbiotic symbionts are separated from free-living symbionts by a host cell, I assumed that the density of symbionts residing host cells does not affect the death of free-living ones. Note that by setting $b_0 = 0$, my model can include the case where the symbiosis is obligate for symbionts. (iii) *Infection of free-living symbionts to free-living hosts*. A free-living symbiont can infect a free-living host at a rate c. (iv) *Cell division and death of endosymbionts*. After infection, an endogenous symbiont proliferates in a host cell with the division rate b and dies in a host cell at a rate d. This endosymbionts' cell division rate b controlled by symbionts themselves and their death rate d controlled by hosts are two key traits focused in my evolutionary analysis. (v) *Host cell burst by overgrown symbionts*. If the number of endogenous symbionts exceeds a prefixed threshold (2 in this model), the host cell is assumed to burst and the symbionts are released either alive (with probability q) or dead (with probability $1 - q$). (vi) *Cell division and natural death of symbiotic hosts*. In addition to the burst, the death not induced by the burst (hereafter, called natural death) also occurs at a rate $\widetilde{D} = D \sum_{i=0}^{2} H_i$, where, as in free-living hosts, the host natural mortality is proportional to the total host density with a proportionality constant

D. The endogenous symbionts can survive the natural death of their hosts with probability p. Finally, a symbiotic host (a host harboring a symbiont or two) proliferates at a cell division rate B, and upon the division each symbiont in the mother cell is randomly and independently distributed in either of the two daughter cells. Combining these processes, the host densities H_0, H_1, H_2 and the free-living symbionts density S change with time as

$$\begin{aligned}
\frac{dH_0}{dt} &= \left(B_0 - \widetilde{D}_0\right)H_0 - cSH_0 + (B+d)H_1 + \frac{B}{2}H_2, \\
\frac{dH_1}{dt} &= cSH_0 - \left(\widetilde{D} + b + d\right)H_1 + (B+2d)H_2, \\
\frac{dH_2}{dt} &= bH_1 - \left(\frac{B}{2} + \widetilde{D} + 2b + 2d\right)H_2, \\
\frac{dS}{dt} &= \left(b_0 - \tilde{d}_0\right)S - c\,H_0S + p\,\widetilde{D}H_1 + \left\{2p\,\widetilde{D} + 6\,q\,b\right\}H_2,
\end{aligned} \tag{1}$$

where six of the last term in the fourth equation comes from the division rate per a host cell harboring *two* symbionts, $2b$, times the number of symbionts released by the burst consisting of the two symbionts and the one offspring reproduced on the division (see Figure 3.1 that illustrates each transition of states, and Table 3.1 for the definition of symbols). In Discussion, I will comment on the alternative case where the accumulation of symbionts decreases the host fitness gradually rather than it bursts the host at a specific threshold.

I also assume that both hosts and symbionts can benefit from their symbiotic interaction through the enhancement of their own survival. I measure the magnitude of these benefits by the reduction of the death rates in the symbiotic state compared to that in the free-living state (D_0/D for hosts and d_0/d for symbionts); the larger are these ratios, the greater are the reduction in death rates by virtue of symbiotic interaction. It should be noted here, however, that the interaction is not always mutualistic even if

hosts can enjoy the benefit of reduced mortality ($D_0/D > 1$). Indeed, if symbionts divide too rapidly within a host cell, the burst of the host cell occurs very frequently, and its cost for hosts may exceed the benefit of reduced mortality brought by symbiosis – such symbionts are parasitic and harmful for hosts. Therefore, the mode of the symbiotic interaction depends not only on the magnitude of reduction in mortality but also on the burst rate of host cells by over-grown symbionts.

The cell division rate of endogenous symbionts and the host generosity (host-controlled survivorship of endogenous symbionts) focused in my model thus describe the continuum of host-symbiont interaction connecting parasitism and mutualism, and also define a tradeoff between horizontal and vertical transmission. If symbionts in a host cell divide faster than their host, symbionts are likely to burst their host cell and thus tend to be transmitted horizontally. On the other hand, if symbionts divide slowly, symbionts tend to remain in their host cell and thus are likely to be transmitted vertically. Therefore, symbionts face the tradeoff between horizontal and vertical transmission, and they can choose either of transmission modes by changing their division rate within a host cell.

3.2.2. Coevolutionary dynamics of symbionts and hosts

I analyzed coevolutionary dynamics of symbionts and hosts by considering the invasibility of their mutants in the equilibrium population of residents. Suppose that the populations of hosts and symbionts reach an equilibrium state, where I represent the densities at the equilibrium by symbols with hats: $\widehat{H}_0, \widehat{H}_1, \widehat{H}_2$ and $\hat{S}$. I then ask whether a mutant symbiont can invade the equilibrium population of resident symbionts.

I define the invasion fitness $w(b',b)$ of the mutant as the basic reproductive ratio of a symbiont residing a host cell, where b' and b respectively are mutant's and resident's cell division rates in a host cell. A derivation in Appendix A shows that

$$w(b',b) = w_{\mathrm{VT}}(b',b) + \alpha(b) w_{\mathrm{HT}}(b',b), \tag{2}$$

where the invasion fitness is represented as a sum of the two components of basic reproductive ratios through vertical and horizontal transmission, $w_{\mathrm{VT}}(b',b)$ and $\alpha(b)w_{\mathrm{HT}}(b',b)$ formally defined later. The component of basic reproductive ratio through horizontal transmission is further decomposed into the expected number of free-living symbionts produced by a symbiotic host, $w_{\mathrm{HT}}(b',b)$, and the expected number of symbionts produced in newly infected hosts from a released free-living symbiont, $\alpha(b)$. Note that, because the basic reproductive ratio of a resident, $w(b,b)$, is unity at the equilibrium, $w_{\mathrm{VT}}(b,b)$ represents the proportion of the contribution of vertical transmission to the whole reproduction. These fitness components are expressed as

$$\begin{aligned} w_{\mathrm{VT}}(b',b) &= \frac{b'}{\widehat{D}+b'+d}\frac{B+2d}{\frac{1}{2}B+\widehat{D}+2b'+2d} \\ w_{\mathrm{HT}}(b',b) &= \frac{p\widehat{D}}{\widehat{D}+b'+d} + \frac{b'}{\widehat{D}+b'+d}\frac{2p\widehat{D}+6qb'}{\frac{1}{2}B+\widehat{D}+2b'+2d}, \\ \alpha(b) &= \frac{c\widehat{H}_0}{\hat{d}_0 + c\widehat{H}_0 - b_0}, \end{aligned} \tag{3}$$

where $\widehat{D}$ is the death rate of symbiotic hosts at the equilibrium ($\widehat{D} = D\sum_{i=0}^{2}\widehat{H}_i$) and $\hat{d}_0$ is the death rate of free-living symbionts at the equilibrium ($\hat{d}_0 = d_0\hat{S}$) (see Appendix A for derivation).

I next define the invasion fitness $W(d',d)$ of a mutant host that adjusts their symbionts' mortality to rate d' in the equilibrium population of resident host that

adjusts the symbiont mortality to d, in a similar way as I defined the symbionts invasion fitness (2), and thus, it can be described as follows:

$$W(d', d) = W_{\mathrm{VT}}(d', d) + A(d)W_{\mathrm{HT}}(d', d), \qquad (4)$$

where the first and second terms correspond to the reproduction through the routes of vertical and horizontal transmission, respectively. These terms are given as follows:

$$\begin{aligned} W_{VT}(d', d) &= \frac{b}{\widehat{D} + b + d'} \frac{B + 2d'}{\frac{1}{2}B + \widehat{D} + 2b + 2d'}, \\ W_{HT}(d', d) &= \frac{B + d'}{\widehat{D} + b + d'} + \frac{b}{\widehat{D} + b + d'} \frac{\frac{1}{2}B}{\frac{1}{2}B + \widehat{D} + 2b + 2d'}, \\ \mathrm{A}(d) &= \frac{c\hat{S}}{\widehat{D}_0 + c\hat{S} - B_0}, \end{aligned} \qquad (5)$$

where $\widehat{D}_0$ is the death rate of free-living hosts at the equilibrium ($\widehat{D}_0 = D_0 \sum_{i=0}^{2} \widehat{H}_i$) (see Appendix A). The equilibrium population densities of residents in the expressions of invasion fitness Eq. (3.2)-(3.5) are calculated numerically by equating the left hand sides of Eq. (3.1) to zero.

According to Dieckmann and Law (1996), the long-term coevolutionary dynamics of symbionts and hosts can be represented by using the invasion fitness as following:

$$\dot{b} = \theta \left. \frac{\partial w(b', b)}{\partial b'} \right|_{b'=b}, \qquad (6a)$$

$$\dot{d} = \Theta \left. \frac{\partial W(d', d)}{\partial d'} \right|_{d'=d}, \qquad (6b)$$

where the dots indicate the time derivative in an evolutionary time scale, θ and Θ are parameters of symbionts and hosts that determine the speed of their evolution, which consist of the rate of mutation and the variance of its phenotypic effect. Because θ and

Table 3.1. List of symbols in the population dynamics model.

symbol	definition
H_i	density of hosts having i symbionts inside of them (0 means free-living)
S	density of free-living symbionts
B_0	birth rate of a free-living host per capita per unit time
$\widetilde{D}_0$	death rate of a free-living host per capita per unit time, which is proportional to the total density of hosts, $\widetilde{D}_0 = D_0 \sum H_i$, where D_0 is constant
B	birth rate of a symbiotic host per capita per unit time
$\widetilde{D}$	death rate of a symbiotic host per capita per unit time, which is proportional to the total density of hosts, $\widetilde{D} = D \sum H_i$, where D is constant
b_0	birth rate of a free-living symbiont per capita per unit time
$\tilde{d}_0$	death rate of a free-living symbiont per capita per unit time, which is proportional to the density of free-living symbionts, $\tilde{d}_0 = d_0 S$, where d_0 is constant
b	birth rate of a symbiont inside a host per capita per unit time
d	birth rate of a symbiont inside a host per capita per unit time
c	association rate per contact between a free-living host and symbiont per unit time
p	probability that a symbiont survives the natural death of its host
q	probability that a symbiont survives the burst of its host

Θ are always positive, the signs of the derivatives of the invasion fitness determine the direction of evolution.

3.3. Results

I will first show the results for the case where either symbionts' or hosts' trait evolves while the other remains unchanged, in order to clarify the impact of key parameters on the evolutionary dynamics. I will then show the results of joint evolution of symbionts' and hosts' traits.

3.3.1. Evolution of self-limited cell division of symbionts

I first study the case where the symbiont evolves its division rate in host cells but hosts' generosity does not evolve. The evolutionary dynamics of the symbiont's trait is described by Eqs. (6a). I mainly focused on the end point of this evolutionary dynamics of the division rate of symbionts (strictly speaking, the stability of an equilibrium of this dynamics only guarantees its "convergence stability", but I also numerically confirmed its "evolutionarily stability" by calculating the second derivative of the invasion fitness, $\partial^2 w(b', b)/\partial b'^2$). First, I will show the results for the case where symbionts can survive the burst and natural death of their host with the same probability (i.e. $q = p$). I will then study the effect of difference in these probabilities.

Figure 3.2a shows the evolved division rate of symbionts in a host when $q = p$. A low division rate of symbionts, and hence a large dependence on vertical transmission evolves if not only symbionts but also hosts can enjoy large benefit from their symbiotic interaction (Fig. 3.2b). Even if symbionts can obtain large benefit from

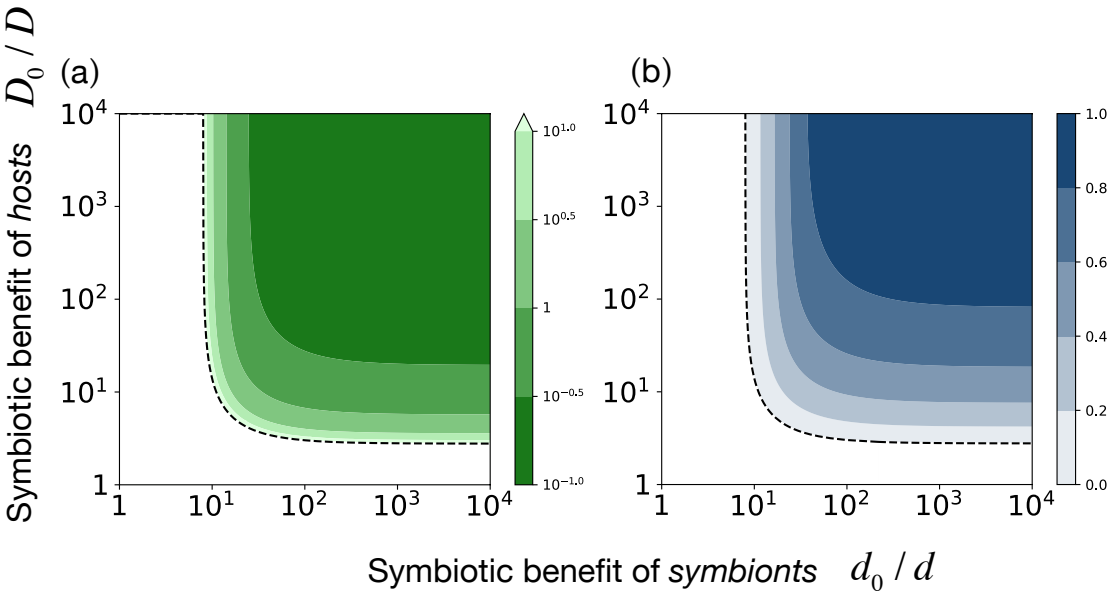

Figure 3.2. The effect of symbiotic benefits of hosts and symbionts on the evolution of symbionts. Panel (a) shows the heat map for the evolved value of division rate of symbionts b. A lighter color in the panel indicates a higher value of the evolved division rate b. In particular, b evolves to infinity in the white region below the dashed line. In each axis, the death rates of the symbiotic state are varied, while those in the free-living state are kept constant. Panel (b) shows the heat map for the proportion of vertical transmission of symbionts when the division rate of symbionts b is reached to the evolutionary end point shown in panel (a). A darker color indicates that symbionts rely more heavily on vertical transmission. The white region below the dashed line, symbionts rely perfectly on horizontal transmission. Parameter values are $B_0 = B = 1$, $D_0 = 0.5, b_0 = 3, d_0 = 2, c = 0.1$, and $p = q = 1$.

the symbiosis with their hosts, symbionts evolutionarily increase their division rate if hosts enjoy only small symbiotic benefit. Leaving the discussion on the reason why this paradoxical result follows to the next paragraph, I here only summarize its evolutionary consequence: if only symbionts can enjoy sufficiently large benefit from symbiotic interaction, horizontal transmission of symbionts via host's burst becomes frequent (Fig. 3.2b). In particular, if either symbiotic benefit of symbionts or that of hosts is too low (the white region in Fig. 3.2a), the division rate of symbionts increases without limit.

These results are essentially unaffected by the change of the survival probabilities p and q as long as $p = q$ (Fig. 3.3). One may expect that symbionts should reduce their division rate if symbionts almost always die when their host dies ($p \approx 0$), because bursting their host inevitably kills themselves. However, my result shows that this is not the case; even in such a situation the division rate of symbionts can evolutionarily increase. On the other hand, when surviving the burst of their host is much more difficult for symbionts than surviving its natural death (i.e. $q \ll p$), the low division rate can evolve in broader range of symbiotic benefits than when q and p are similar (compare Fig. 3.4a with Fig. 3.2a). However, note that in contrast to the previous results, symbionts do not always rely on vertical transmission even if they divide slowly (Fig. 3.4b). When the benefit of hosts is low (i.e. the death rate of symbiotic hosts is high), symbionts evolve to divide slowly but they are almost always transmitted horizontally through the natural death of their host.

In summary, symbionts can evolve to limit their cell division (i) when the mutualistic interaction of hosts and symbionts brings large benefits to both of them or (ii) when surviving the burst of their host is much more difficult for symbionts than surviving its natural death. However, the second condition leads to the low division rate

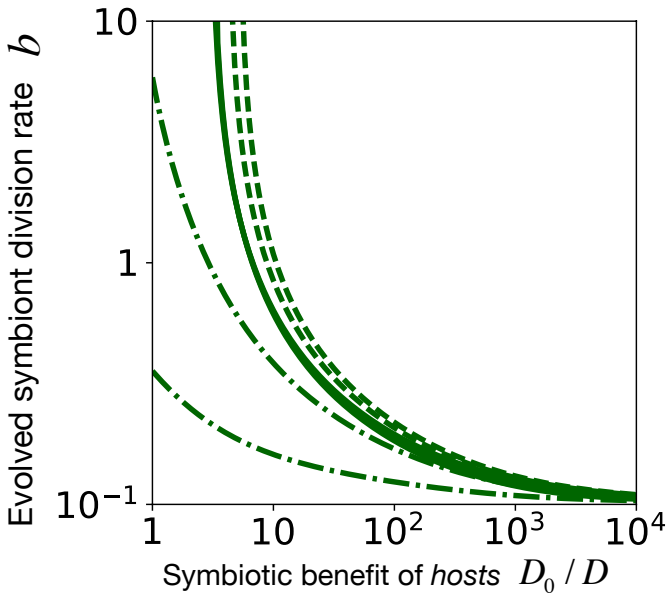

Figure 3.3. The effect of the probability of symbionts surviving the death of their host on the evolution of division rate of symbionts. Dot dashed, solid, and dashed lines respectively indicate the evolved division rate when $p > q$, $p = q$, and $p > q$, where p and q are the probability of symbionts surviving the natural death and burst of their host. In order starting from the bottom, the values of (p, q) are $(1,0.1)$, $(1,0.5)$, $(1,1)$, $(0.5,0.5)$, $(0.1,0.1)$, $(0.5,1)$, and $(0.1,1)$, respectively, where three solid lines for the case $q = p$ are almost identical and hard to be distinguished with each other The other parameter values are $B_0 = B = 1$, $D_0 = 0.5, b_0 = 3, d_0 = 2$, $d = 0.02$, and $c = 0.1$. In changing D_0/D in the horizontal axis, the death rate in the symbiotic state D is varied, while that of free-living state D_0 is kept constant.

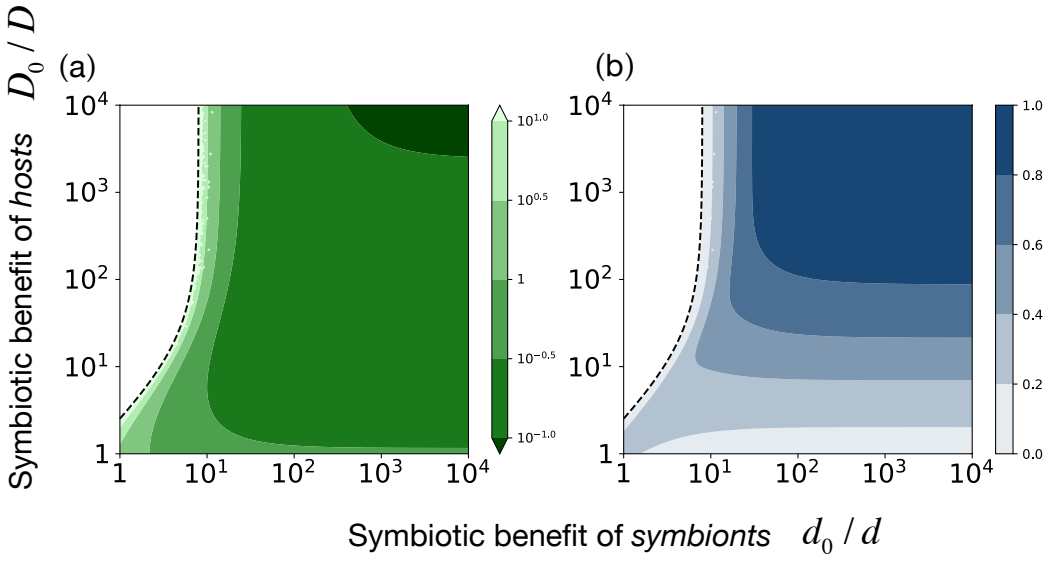

Figure 3.4 The effect of symbiotic benefits of hosts and symbionts on the evolution of symbionts under the low probability of symbionts surviving the burst of the host. Colors of panel (a) and (b) are the same as in Fig. 3.2. All the parameters except the probability of symbionts surviving the burst, q, now set to 0.1, are the same as in Fig. 3.2.

of symbionts but not to vertical transmission. Therefore, the first condition is required in order that symbionts evolve to rely almost exclusively on vertical transmission.

3.3.2. Evolutionary maintenance of symbionts by generous hosts

I next study the opposite case where symbionts' trait does not evolve but hosts' does. The evolutionary dynamics is described by Eq. 6b. The end point of the evolutionary dynamics corresponds to the evolved host generosity (Fig. 3.5), which shows that the host evolves to be generous (maintains their symbionts for a long time) only if symbionts divide slowly. In other words, for mutualistic symbionts that rarely burst their host, the host generosity evolves. Conversely, if symbionts divide quickly and thus burst their host frequently, hosts become less generous and resist those virulent symbionts by killing them rapidly.

3.3.3. Coevolutionary dynamics of hosts and symbionts

Finally, I study coevolution of symbionts and hosts. The coevolutionary dynamics of division rate of symbionts and generosity of hosts (Eq. 6) are described by combining the evolutionary dynamics for each. Here, I only focus on the case $q = p$ because under the case $q \ll p$, the evolved division rate is almost always low and essentially unaffected by the benefit of symbionts, or host generosity. Figure 3.6 shows typical phase planes of the coevolutionary dynamics. When the death rate of symbiotic hosts, D, is not well reduced from that of free-living hosts, D_0 (that is, when the symbiotic benefit of hosts is low), there is no joint evolutionary equilibria for symbionts' and hosts' traits (Fig. 3.6a). The division rate of symbionts increases without limit while the

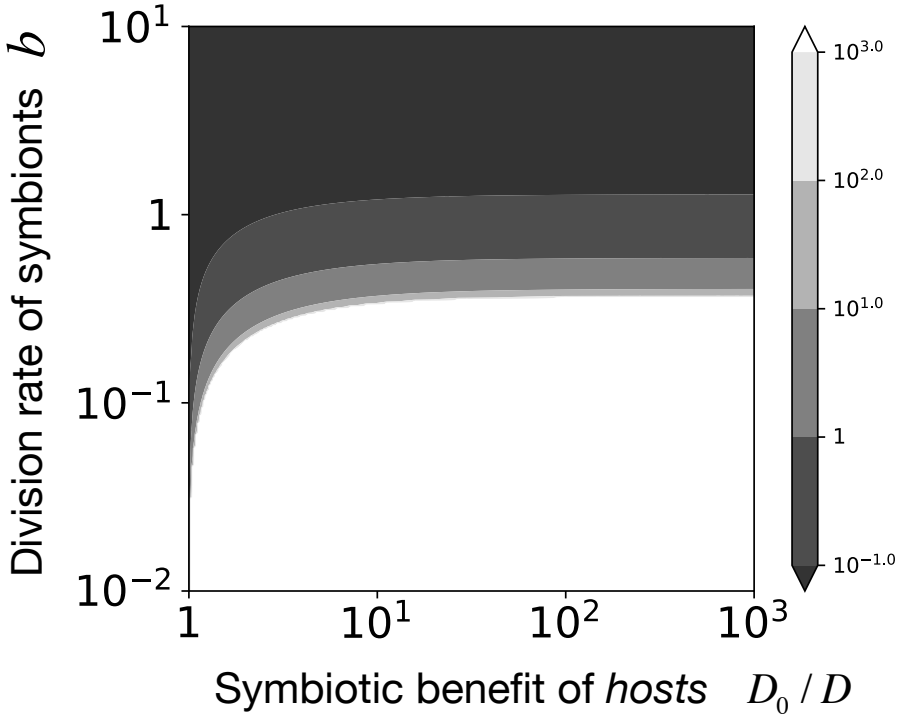

Figure 3.5. The effect of symbiotic benefit of hosts and division rate of symbionts on the evolution of host generosity. The darker color in the panel indicates a higher value of the evolved generosity of hosts (i.e. the death rate of symbionts residing the host cell, d). As previous figures, in changing D_0/D in the horizontal axis, the death rate in the symbiotic state D is varied, while that of free-living state D_0 is kept constant. Parameter values are $B_0 = B = 1$, $D_0 = 0.5$, $b_0 = 3$, $d_0 = 2$, $c = 0.1$, and $p = q = 1.0$.

host generosity decreases without limit, leading to arms race between them. The symbionts are better off by dividing themselves as rapidly as possible and they rely exclusively on horizontal transmission for their reproduction, as lytic parasites do. The hosts are better off by being intolerant as much as possible to symbionts and by eliminating them rapidly. On the other hand, if the death rate of hosts becomes much smaller by symbiosis, $D \ll D_0$ (that is, when the symbiotic benefit of hosts is high), in addition to the aforementioned end point of parasitism, another coevolutionary outcome arises in which symbionts self-limit their division rate and are transmitted mainly vertically and hosts remain generous so that they keep their symbionts for a long time (Fig. 3.6b). When the latter coevolutionary outcome occurs, the interaction between hosts and symbionts is mutualistic, and symbionts behave like organelles, which benefit their host and are transmitted vertically.

Whether host-symbiont relationship coevolves towards the parasitism or vertically transmitted mutualism depends on the initial condition of the coevolution. For example, if the relationship between symbionts and hosts originates from prey–predator relationship, where a host (a predator) is not generous and attempts to digest its symbionts (preys) rapidly and symbionts almost cannot divide within the host (high d and low b, corresponding to the left bottom region of Fig. 3.6b), vertically transmitted mutualism can be achieved (i) if symbionts evolve much slower than hosts or (ii) if symbionts have digestion resistance or a certain indigestibility in advance. The condition (i) means that the coevolutionary trajectory moves towards right rapidly but moved upward slowly in Fig. 3.6b, until hosts and symbionts coevolve to vertically transmitted mutualism. The condition (ii) corresponds to that the initial state of coevolution is shifted from a left bottom position to right in Fig. 3.6b just after the

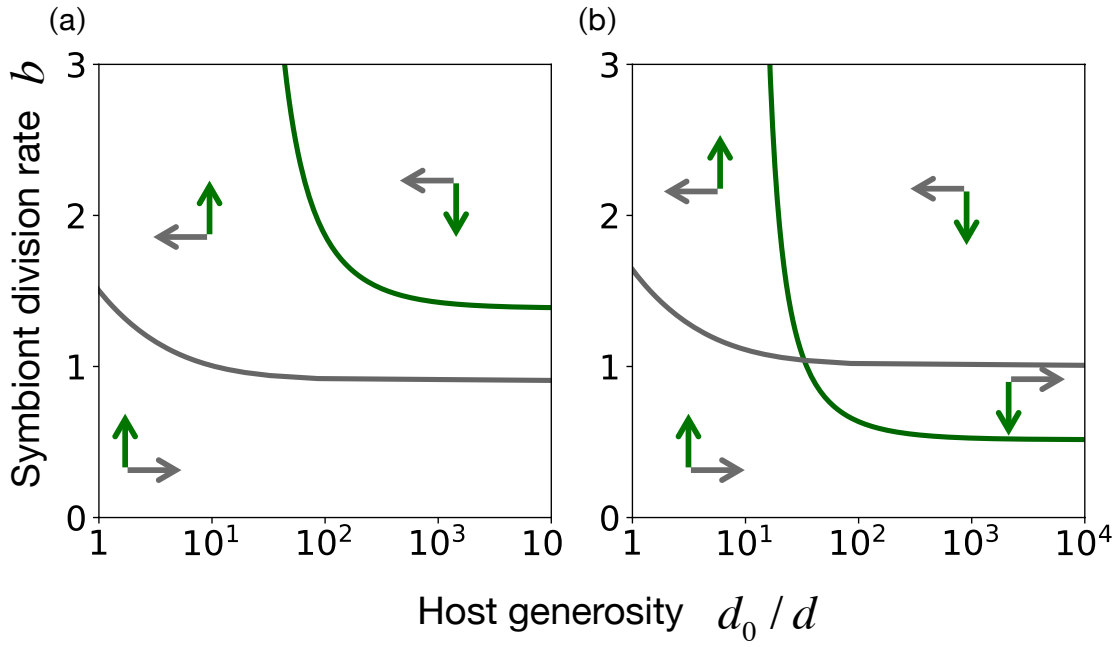

Figure 3.6. The coevolutionary dynamics of division rate of symbionts and generosity of hosts. (a) and (b) are typical cases where the symbiotic benefit of hosts is low, $\log_{10}(D_0/D) = 0.68$, and high (=1.0), respectively. Arrows and lines indicate the directions and nullclines of evolutionary dynamics, respectively. The dark green and gray arrow respectively corresponds to the direction of the change in symbiont controlled trait b (cell division rate of endosymbiont) and host controlled trait d_0/d (generosity) in coevolutionary dynamics. The other parameters are $B_0 = B = 1$, $D_0 = 0.5$, $b_0 = 3$, $d_0 = 2$, $c = 0.1$, and $p = q = 1$.

acquisition of anti-predatory resistance in prey. Conversely, if the relationship originates from a naïve host–parasite system, where symbionts (parasites) divide within a host very rapidly and a naïve host without any resistance mechanisms is generous for them (the right top region of Fig. 3.6b), vertically transmitted mutualism can be achieved if symbionts evolve much faster than hosts (then, the coevolution goes down rapidly and thus turns to the right by going below the gray line in Fig. 3.6b). Note that in the case $D \ll D_0$, parasites benefit their host but proliferate so rapidly that the net effect on the host is negative. In other words, if victims of interspecific interaction (preys in a prey–predator system and hosts in a host–parasite) can evolve faster than their exploiters (predators and parasites), preys move from the bottom-left (prey-predator relation) to the top-left corner (the arena for host-parasite arms race), and hosts move from the top-right (naïve host-parasite relationship) to the left-middle region (mutualistic relationship) in Fig. 3.6b. It means that the coevolution leads to resistance against their exploiters, increases exploitation further to overcome the resistance, and eventually induces an arms race.

3.4. Discussion

In this study, I theoretically revealed the condition under which symbionts evolve to self-limit their cell division to keep pace with their host under the tradeoff between vertical and horizontal transmissions. My study has shown that although it may look paradoxical at first glance, the synchronized cell division can be achieved (i) when the mutualistic interaction of hosts and symbionts brings large benefits to both of them or (ii) when surviving the burst of their host is much more difficult for symbionts than surviving its natural death. In particular, the condition (i) is required for symbionts to be

relying on vertical transmission. Thus, symbiogenesis can proceed under an already mutualistic relationship where symbionts are willing to reduce their division rate.

The reason why the benefit of *hosts* plays a critical role in the evolution of limited cell division of *symbionts* in my model can be interpreted as follows. If the symbiotic benefit of hosts is not sufficiently large, free-living hosts are relatively abundant, and hence, finding new hosts becomes easier for a released symbiont than when benefit of hosts is high. This will make a faster cell division rate more beneficial for symbionts than when hosts enjoy large symbiotic benefit. Moreover, the natural death of hosts occurs frequently when the symbiotic benefit is low, which will mask the risk of bursts by over-grown endosymbionts, making a higher division rate beneficial again. These two factors associated with low symbiotic benefit for hosts can promote the evolution towards larger weight for horizontal transmission at the expense of reduced vertical transmission. These results are essentially unaffected by the change of the survival probabilities p and q as long as $p = q$. It can be considered as follows. Suppose that p (=q) is small. Since symbionts almost always die upon the host's death, they suffer from the burst of their host. However, once they survive host's death, the competition for acquiring new free-living hosts is weak because almost all symbionts, their potential competitors, die with host's death. In this way, the difficulty of surviving host's death will be canceled out by a large benefit of easily finding new hosts.

This condition for the evolution of vertical transmission needed in my model is severer than that of previous studies. For example, in the model of Yamamura (1996), symbionts always evolve to increase their vertical transmission because horizontal transmission is not reduced at the cost of vertical transmission. Similarly, organisms can evolve to rely exclusively on vertical transmission regardless of partner's benefits when

transmission mode does not affect their fecundity (Law & Dieckmann, 1998). Moreover, even if reducing virulence makes vertical transmission effective but decrease horizontal transmission rate, pathogens usually evolve to rely on both transmission modes without mutualistic benefits (Sasaki & Iwasa, 1991; Frank, 1996b; Van Baalen, 2000; Day, 2001; Ebert, 2013). On the other hand, symbionts evolve to rely exclusively on horizontal transmission when host's benefit is absent. The necessity of mutualistic benefit in my model will result from that symbionts are released and spread horizontally by killing their host. Under the situation, the more rapidly symbionts divide, they can improve horizontal transmission more. Thus, vertical transmission must be very efficient to suppress the selective force favoring horizontal transmission, and thus the large benefit of hosts is required for the suppression as explained above.

Various symbionts can actually be released on the death of their host. An example is parasitic bacteria *Holospora*, which infect paramecium ciliates and rely on both horizontal and vertical transmission (Fujishima, 2009). However, they usually proliferate faster than their host and accumulate so much that it disturbs host's cell division and kills the host (Fujishima, 2009). As a result, they are released on the death of the host and, they rely mainly on transmitted horizontally. Because they do not benefit their host (except under sever environment; Fujishima (2009)), this example supports my theoretical prediction. In this way, my model indicates that the evolution of self-limited cell division will be more difficult than previously thought. On the other hand, my model also suggests that even if the tradeoff potentially allows such an infinite division rate, self-limited division can be achieved as long as the symbiotic benefits of hosts and symbionts are large enough. In fact, mutualistic algae *Chlorella*, which associates with *Paramecium brusca*, can also be released on the death of the host

(Omura *et al.*, 2004) but divide slowly to keep pace with the host (Kadono *et al.*, 2004; Takahashi *et al.*, 2007). Although it is unclear whether their mutualism was established in advance and drove the evolution of synchronized cell division, the mutualism probably contributes at least to the evolutionary maintenance of synchronized cell division.

Conventionally, vertical transmission has been considered to be important for resolving conflicts between hosts and symbionts and for maintaining mutualism evolutionarily, because such vertical transmission brings the interests of a host and its symbionts into line (Fine, 1975; Yamamura, 1996; Sachs *et al.*, 2004). Thus, the evolutionary transition from parasitism to mutualism, which is driven by vertical transmission, is commonly hypothesized (Yamamura, 1996; Moran & Wernegreen, 2000; Sachs & Simms, 2006). However, my model has demonstrated the opposite dependency that mutualistic benefit is the prerequisite for the evolution of vertical transmission. Phylogenic studies of endosymbiosis in various taxa have suggested that the evolutionary transitions between parasitism and mutualism are rare (Moran & Wernegreen, 2000; Sachs & Simms, 2006; Sachs *et al.*, 2011)and that vertically transmitted mutualism usually originated from a free-living state via horizontally transmitted mutualism (Sachs *et al.*, 2011), although they do not include unicellular hosts as I mainly focused on. Therefore, the evolution of vertical transmission driven by pre-existing mutualism, suggested by my model, might play a critical role in the transition and emergence of vertically transmitted mutualism. In addition, in the mutualism between paramecium and chlorella mentioned above, it is experimentally shown that the ciliates can associate preferentially with photosynthesizing chlorella compared with ones whose photosynthesis is inhibited (Tanaka & Miwa, 1996). The

preference by hosts might promote the evolution of nutrient supply by chlorella as partner choice even if vertical transmission is absent. Therefore, it is possible that the mutualism was established in advance to the evolution of vertical transmission and drove it.

In addition to the condition of the evolution of self-limited division rate, I also found that coevolution of division rate of symbionts and generosity of hosts leads to the host-parasite arms race or vertically transmitted mutualism and that those evolutionary consequences are bistable depending on the initial condition of evolution. In particular, vertically transmitted mutualism can be achieved from a prey-predator system. Such transition corresponds to "stuck in the throat model" (Van Dooren *et al.*, 2001; Inouye & Okamoto, 2005). The bistable outcome means that the evolutionary transition from parasitism to vertically transmitted mutualism is difficult. The opposite transition from mutualism to parasitism is difficult, either. This result is consistent with the phylogenic studies mentioned above (Moran & Wernegreen, 2000; Sachs & Simms, 2006; Sachs *et al.*, 2011). Moran & Wernegreen (2000) argue that the main reason for the difficulty of the transition is that endosymbionts are separated within a host cell and thus cannot obtain gene sets for the evolutionary transitions between parasitism and mutualism through horizontal gene transfer. I have shown another reason for the difficulty of evolutionary transition from parasitism to mutualism from the aspect of evolutionary ecology; even if such constraints on horizontal gene transfer are absent, the evolutionary transition is still difficult because the coevolutionary dynamics is bistable between parasitism and mutualism.

I proposed a simple mathematical model to incorporate the tradeoff between vertical and horizontal transmission through the division rate of symbionts. However, as

I mentioned in the Model selection, there are several limitations in my model.

Firstly, I assumed that a host can keep only up to two symbionts. Although the assumption may be realistic in several mutualisms, for example trypanosome and bacteria, it is by no means general. I expect that increasing host's capacity of symbionts relaxes the tradeoff between vertical and horizontal transmission that symbionts face and increases the evolved division rate of symbionts, because the high capacity allows symbionts to proliferate more rapidly without killing their host. However, my results that the evolution of vertical transmission requires highly beneficial mutualism would still hold because symbionts would face qualitatively the same tradeoff as in the original model.

Secondly, I made a simplistic assumption that over-accumulation of symbionts leads to the burst of their host cell. In a more realistic case, accumulation of symbionts may suppress the intracellular population growth of symbionts and thus the population will reach an equilibrium in the host cell. However, it is plausible that the actual density of symbionts per host cell exceeds the optimal density for the host and thus hosts suffer from the over-accumulation to some extent. Even if symbionts cannot burst their host, symbionts should face qualitatively the same tradeoff as my model assumes as long as the accumulation decreases the growth rate of hosts and as long as symbionts are released upon the death of hosts. Therefore, I believe that my result can provide insights into the evolution of vertical transmission for wider symbiotic systems than what I assumed.

Thirdly, in my model, a host is infected by only one strain of symbionts throughout its symbiotic period, and co-infection or super-infection does not occur. If multiple infection is allowed, I expect that the evolved division rate of symbionts would

increase and thus vertical transmission would be hindered. This is because the competition among coexisting strains in a host would favor more exploitive strains of symbionts (Axelrod & Hamilton, 1981; Bremermann & Pickering, 1983; Sasaki & Iwasa, 1991). Moreover, because super-infection allows symbionts to infect hosts that are infected already, it would facilitate the evolution of horizontal transmission of symbionts (Day & Gandon, 2006)

Finally, I did not assume an evolutionary cost of benefiting symbiotic partners. In particular, I assumed that generosity did not change the fitness of host directly in my model. In reality, however, a generous host may incur the cost of keeping symbionts. An intolerant host may enjoy the benefit from assimilated symbionts. As mentioned above, mutualism and vertical transmission are mutually dependent on each other in the evolution. Therefore, evolution of vertical transmission or mutualism cannot be discussed separately --- their joint evolutionary pathway should define the process of symbiogenesis.

3.5. Appendix

When symbiont mutants are rare, their population dynamics in the equilibrium population, which consists of residents, can be given approximately as follows:

$$\frac{d}{dt}\begin{pmatrix} H'_1 \\ H'_2 \\ S' \end{pmatrix} = J_{mut}\begin{pmatrix} H'_1 \\ H'_2 \\ S' \end{pmatrix},$$

$$J_{mut} = \begin{pmatrix} -\left(\widehat{D} + b' + d\right) & B + 2d & c\,\widehat{H}_0 \\ b' & -\left(\frac{B}{2} + \widehat{D} + 2b' + 2d\right) & 0 \\ p\,\widehat{D} & 2p\,\widehat{D} + 6\,q\,b' & b_0 - \hat{d}_0 - c\,\widehat{H}_0 \end{pmatrix}, \quad \text{(A1)}$$

where S' and H'_i are the densities of hosts that harbor i symbiont mutants and

free-living symbiont mutants, respectively, and b' is the division rate of mutants (b is that of residents). Note that since mutants are rare, their death rates are not $\widetilde{D}$ and $\tilde{d}_0$ but $\widehat{D}$ and $\hat{d}_0$. I can decompose the mutant dynamics, $J_{mut} = F - V$, as follows:

$$F = \begin{pmatrix} 0 & B + 2d & c\,\widehat{H}_0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix},$$
$$V = \begin{pmatrix} \widehat{D} + b' + d & 0 & 0 \\ -b' & \frac{B}{2} + \widehat{D} + 2b' + 2d & 0 \\ -p\,\widehat{D} & -\left(2p\,\widehat{D} + 6\,q\,b'\right) & \hat{d}_0 + c\,\widehat{H}_0 - b_0 \end{pmatrix}, \tag{A2}$$

where F gives the production rate of hosts harboring one symbiont and V corresponds to the rate of the others, the deaths and transitions. Given the decomposition, FV^{-1} corresponds to the next-generation matrix, and thus, the maximum absolute value of all its eigenvalues represents the basic reproductive ratio $\mathcal{R}_0$ (Diekmann & Heesterbeek, 2000; Hurford *et al.*, 2010). They are given as follows:

$$FV^{-1} = \begin{pmatrix} \frac{v_{21}}{v_{11}}\frac{f_{12}}{v_{22}} + \frac{f_{13}}{v_{33}}\left(\frac{v_{31}}{v_{11}} + \frac{v_{21}}{v_{11}}\frac{v_{32}}{v_{22}}\right) & \frac{f_{12}}{v_{22}} + \frac{v_{32}}{v_{22}}\frac{f_{13}}{v_{33}} & \frac{f_{13}}{v_{33}} \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix},$$
$$\mathcal{R}_0 = \frac{v_{21}}{v_{11}}\frac{f_{12}}{v_{22}} + \frac{f_{13}}{v_{33}}\left(\frac{v_{31}}{v_{11}} + \frac{v_{21}}{v_{11}}\frac{v_{32}}{v_{22}}\right), \tag{A3}$$

where f_{ij} and v_{ij} are the ij-components of the matrices F and V, respectively. I denote $\mathcal{R}_0$ by $w(b', b)$ in the main text. The first term, the coefficient of the parentheses, and the parentheses of $\mathcal{R}_0$ are respectively $w_{\mathrm{VT}}(b', b)$, $\alpha(b)$, and $w_{\mathrm{HT}}(b', b)$ in the main text. Because the fitness of a host mutant can be derived by the same manner, I do not show the derivation here.

4. Concluding Remarks and Perspectives

4.1. Concluding remarks

Since mutualism is vulnerable to the spread of free-riders and is likely to easily fall into parasitism, it cannot be evolutionarily achieved and maintained without some mechanisms that encourage cooperation and/or discourage free-riding (Bronstein, 2001b; Wilkinson & Sherratt, 2001; Sachs & Simms, 2006; Akçay, 2015). Thus, in order to understand the evolution of symbiosis, in particular the emergence of mutualism, it is essential to reveal how such mechanisms have been formed evolutionarily. Various mechanisms that enhance mutualism have been proposed, which can be categorized into either discrimination or partner fidelity feedback (Bull & Rice, 1991; Sachs *et al.*, 2004; Foster & Wenseleers, 2006; Frederickson, 2013; Jones *et al.*, 2015). However, the evolution of these mechanisms themselves is theoretically paradoxical.

Discrimination evolves if the mixture of free-riders and cooperators is already present, but cooperators are supposed not to be maintained without discrimination (West *et al.*, 2002; Foster & Kokko, 2006; McNamara *et al.*, 2008). Moreover, if discrimination once spreads in the population its propagation depletes the variability of symbionts, but this variability is the source of selective advantage for discrimination itself, so the success of discrimination ultimately and paradoxically leads to the evolutionary loss of discrimination itself (Foster & Kokko, 2006; McNamara *et al.*, 2008). In chapter 2, I theoretically tried to resolve the paradox by comparing two different types of discrimination as examples: the one-shot and resampling discrimination. As a result, I found that the paradox is not an inherent feature of

evolution of discrimination but a problem specific to a certain model. My results show that the magnitude of selective advantage of one-shot type discrimination is proportional to the frequency of free-riding symbionts, rather than the variability of symbionts, and that one-shot type discrimination can therefore invade the free-rider/non-discriminator population without pre-existing variability of symbionts in the population. Contrary to what was argued in previous studies, my analysis also suggests that the difficulty in maintaining discrimination in previous studies, the domination of discrimination resulting in the destruction of its own selective advantage, was caused not by discrimination *per se* but by their additional assumptions of repeated retaliation between partners, e.g. partner fidelity feedback (Foster & Kokko, 2006). Therefore, without such repeated retaliation, discrimination can be maintained easily even if the supply of variability is absent. In this way, my results clearly reveal that mutualism promoted by discrimination can emerge and persist more easily than previously considered.

Synchronized cell division is a driving force for partner fidelity feedback in mutualism between unicellular hosts and their endosymbionts, because it is a mechanism implementing vertical transmission in the mutualism and leading to a permanent relationship between hosts and symbionts (Kadono *et al*. 2004; Takahashi *et al*. 2007; Motta *et al*. 2010). However, it is apparently paradoxical that symbionts self-limit their own cell division rate and reduce the principal component of their fitness. In chapter 3, I theoretically explored the condition under which symbionts evolve to self-limit their cell division to keep pace with their host's reproduction under the tradeoff between vertical and horizontal transmissions. My study has shown that, although it may look paradoxical at first glance, the synchronized cell division and

vertical transmission can evolve when the mutualistic interaction of hosts and symbionts brings large benefits to both of them. Conversely, if either symbiotic benefit of symbionts or that of hosts is too low, the division rate of symbionts increases unlimitedly, and they ultimately rely almost exclusively on horizontal transmission. Although it has been conventionally hypothesized that the evolutionary transition from parasitism to mutualism is driven by vertical transmission (Yamamura, 1996; Moran & Wernegreen, 2000; Sachs & Simms, 2006), my model has demonstrated the opposite dependency that mutualistic benefits are the prerequisite for the evolution of vertical transmission. Thus, my results suggest that the evolutionary emergence of vertically transmitted mutualism would be more difficult than previously thought.

In summary, I have studied evolutionary dynamics of discrimination (chapter 2) and evolutionary dynamics of vertical transmission (chapter 3) and revealed conditions required for the emergence and maintenance of these mechanisms. These conditions suggest that mutualism promoted by discrimination can emerge and persist in broader conditions than previously considered but that mutualism promoted by vertical transmission through synchronized cell division could have emerged from horizontally transmitted mutualism.

4.2. Perspectives

It has been considered that discrimination is important in horizontally transmitted mutualism while partner fidelity feedback is essential in vertically transmitted mutualism (Sachs *et al.*, 2004). Previous studies suggested that high genetic variability in symbiont's quality is needed for the evolution of discrimination mechanisms (West *et al.*, 2002; Foster & Kokko, 2006; McNamara *et al.*, 2008). However, high genetic variability hinders partner fidelity feedback that maintains mutualism, because it

facilitates competition among symbionts within a host due to reduced relatedness (Frank, 1994, 1996a). Therefore, discrimination and partner fidelity feedback have been considered separately, and the evolutionary transition from horizontally to vertically transmitted mutualism has been expected to be difficult.

However, results in chapter 3 suggest that vertical transmitted mutualism could have originated from horizontally transmitted mutualism rather than from parasitism. It is therefore important to theoretically explain the evolutionary transition from horizontally to vertically transmitted mutualism in spite of the difficulty that discrimination is expected to be evolutionarily lost during such transition. The transition could have been particularly important in the evolutionary process from free-living to endosymbiosis and to organelle. During the evolutionary transition, the maintenance mechanism of mutualism probably switched from discrimination to vertical transmission (partner fidelity feedback), or the two mechanisms become to work together. Thus, the coevolution of discrimination and vertical transmission is an important problem to investigate the evolutionary transition. Moreover, the coevolution of discrimination and vertical transmission, in particular discrimination in vertically transmitted mutualism, is important not only in explaining the emergence of vertically transmitted mutualism but also in understanding further evolution towards organelles. It is well known that hosts maintain the function of mitochondria by breaking down the defectives preferentially (Ashrafi & Schwarz, 2013). Such a mechanism, called quality control mechanism, functions similarly to discrimination, so studying coevolution of discrimination and vertical transmission can provide insights to the evolution of quality control mechanism as well.

My model of one-shot discrimination can potentially connect horizontally and

vertically transmitted mutualism because a high mutation rate is not required for this discrimination mechanism to work. Moreover, since mutualism and vertical transmission are considered to be mutually dependent on each other in the course of evolution, they might have coevolved by facilitating their evolution mutually, even if relying heavily on vertical transmission can lead to the evolutionary loss of discrimination. Therefore, their joint evolution should become a next central problem in the evolution of symbiosis and symbiogenesis, and I believe that unifying my models in chapters 2 and 3 can provide a theoretical framework to tackle such a complicated evolutionary transition.

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