Sanctions, partner recognition, and variation in mutualism

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Abstract

Mutualistic interactions can be stabilized against invasion by non-cooperative individuals by putting such "cheaters" at a selective disadvantage. Selection against cheaters should eliminate genetic variation in partner quality — yet such variation is often found in natural populations. One explanation for this paradox is that mutualism outcomes are determined not only by responses to partner performance, but also by partner signals. Here, we build a model of coevolution in a symbiotic mutualism, in which hosts' ability to sanction non-cooperative symbionts and ability to recognize symbiont signals are determined by separate loci, as are symbionts' cooperation and expression of signals. In the model, variation persists without destabilizing the interaction, in part because coevolution of symbiont signals and host recognition is altered by the coevolution of sanctions and cooperation, and vice-versa. Individual-based simulations incorporating population structure strongly corroborate these results. The dual systems of sanctions and partner recognition converge toward conditions similar to some economic models of mutualistic symbiosis in which hosts offering the right incentives to potential symbionts can initiate symbiosis without screening for partner quality. These results predict that mutualists can maintain variation in recognition of partner signals, or in the ability to sanction non-cooperators, without destabilizing mutualism, and reinforce the notion that studies of mutualism should consider communication between partners as well as the exchange of benefits.

Introduction

Mutually beneficial interactions between species pose two related conundrums. First, how are mutualisms maintained in the face of the apparent advantages to individuals who accept resources or services but provide none in return? And second, given a mechanism that prevents the evolution of non-cooperative participants, why do members of interacting species vary in mutualistic quality?

The first conundrum may be solved through selective dynamics that offset cheaters' advantage, including partner choice, sanctions, or partner fidelity feedback. Partner choice allows individuals to avoid or discontinue interaction with cheaters (Trivers 1971; Axelrod and Hamilton 1981; Foster et al. 2006), whereas sanctions permit them to cut off or reduce rewards provided (Bull and Rice 1991; West et al. 2002a; West et al. 2002b; Sachs et al. 2004; Akçay and Simms 2011). By contrast, in partner fidelity feedback, cooperative partners receive greater rewards without any active "decision" by the reward-providing species, simply because healthy hosts produce more rewards (Doebeli and Knowlton 1998; Weyl et al. 2010) or because rewards are only accessible to cooperative partners (Archetti et al. 2011a; Archetti et al. 2011b). Each of these mechanisms ensure that non-cooperators are at a long-term fitness disadvantage even if they have an advantage over cooperators in the short term.

Such anti-cheating mechanisms have been found in many mutualisms. Soybean plants can cut off resources to root nodules in which rhizobial bacteria do not fix nitrogen (Kiers et al. 2003) and can scale these sanctions quantitatively to reduce support for less-productive nodules (Kiers et al. 2006). In the obligate brood pollination mutualisms of yuccas and figs, host plants abort flowers to prevent over-exploitation by seed-feeding brood pollinators (Pellmyr and Huth 1994; Jandér and Herre 2010). Reduced growth of ant domatia on herbivore-damaged branches of ant-protected shrubs may be a sanctioning response to poor protection by ants (Edwards et al. 2006a), or may be an example of partner fidelity feedback (Weyl et al. 2010).

These solutions to the first conundrum of mutualism create the second conundrum. Partner choice, sanctions, and partner fidelity feedback should all lead to fixation of cooperative genotypes (Axelrod and Hamilton 1981; Doebeli and Knowlton 1998; West et al. 2002a; West et al. 2002b). Similarly, if interacting mutualists maximize their own fitness by matching each other, coevolution should reduce diversity in both interacting species (Kiester et al. 1984; Kopp and Gavrilets 2006; Yoder and Nuismer 2010). Nevertheless, genetic variation in partner quality is widely observed in natural populations of mutualists (Heath and Stinchcombe 2014), including in many interactions where mutualism-stabilizing mechanisms have been studied directly, such as rhizobial bacteria

and their legume hosts (Simms and Taylor 2002; Heath and Tiffin 2009), mycorrhizal fungi (Hoeksema 2010), ant bodyguards (Ness et al. 2006), and brood pollinators (Pellmyr and Huth 1994; Herre and West 1997; Holland et al. 1999).

Coevolutionary dynamics that can maintain genetic variation in interacting species are known — not from mutualism, but from antagonistic interactions. Host-parasite interactions or competition can either create frequency dependent selection on interacting species (e.g., Dieckmann et al. 1995; Agrawal and Lively 2002), or select for one species to be less well matched to the other (Sasaki 2000; Nuismer and Otto 2005; Kopp and Gavrilets 2006; Yoder and Nuismer 2010). These dynamics are well documented in biological systems in which host defensive responses are activated by recognition of molecules expressed by parasites or pathogens (reviewed by Dybdahl et al. 2014; Nuismer and Dybdahl 2016).

Mutualistic interactions also require processes of recognition and signal exchange. Many brood pollinators respond to complex, host-species-specific floral scents that are not directly related to rewards offered by hosts (Svensson et al. 2005; Okamoto et al. 2007; Soler et al. 2011; Svensson et al. 2016). Host plant volatiles also guide the colonizing queens of plant-protecting ant species and direct the activity of ants' patrols (Edwards et al. 2006a; Edwards et al. 2007; Schatz et al. 2009). Legumes recognize and respond to signals and identifying surface proteins expressed by rhizobia and mycorrhizal fungi (Oldroyd et al. 2011). Immune recognition responses help determine the assembly of animals' microbiomes (Pflughoeft and Versalovic 2012; Cullender et al. 2013; Mutlu et al. 2014). Such signaling and recognition factors in interacting mutualists may coevolve in very different ways from traits governing mutualistic performance, and coevolution of signals and responses to them may affect the coevolution of benefits exchanged.

We hypothesize that coevolving partner communication maintains variation in mutualism outcomes even as sanctions prevent the breakdown of mutualism. Here, we test this hypothesis with models of a mutualism in which outcomes are determined by (1) sanctions against non-cooperative individuals, (2) recognition of signals that are separate from symbiont quality, and (3) sanctions against non-cooperators paired with recognition of signals. We first present analytic models of allele frequency dynamics within a population of two interacting species, then use individual-based coevolutionary simulations to examine a wider range of parameters, and to examine how coevolution in the different models shapes geographic variation as well as local diversity. We find that sanctions alone maintain mutualism without variation, while recognition alone maintains variation but not the mutualism. Incorporating both sanctions and recognition can maintain the mutualism as well as variation in the outcomes of mutualists' interactions.

Methods

We model a mutualism with outcomes determined by sanctions against non-cooperative partners, by recognition of partner signals separate from cooperation, or by both sanctions and recognition. The model is inspired by symbiotic mutualisms such as brood pollination (Pellmyr and Huth 1994; Jandér and Herre 2010) or nutrient symbioses like the legume-rhizobium mutualism (Kiers et al. 2003), in which partly or fully free-living individuals of one species perform a service for members of another species, which provides rewards in return. As in many biological systems the first of these species, the symbiont, is the one considered a potential "cheater," and the host exerts sanctions against such individuals. We consider that "sanctions" refer to any response by hosts such that they pay a reduced cost of hosting a symbiont that provides no benefit, and deny the full benefit of symbiosis to that non-cooperator. Many authors have used "partner choice" to refer to mutualists ceasing interaction with non-cooperative individuals (e.g., Trivers 1971; Axelrod and Hamilton 1981; Foster et al. 2006); we consider this a form of sanctions (following, e.g., Kiers et al. 2003; Segraves 2003; Jander and Herre 2016).

In all three models, we assume that hosts and symbionts encounter each other at random and interact, and that each species i receives a benefit B_i and pays a cost C_i of interaction. For both host and symbiont we assume that fitness is equal to $1 + P_{jk}$, where P_{jk} is the payout (i.e., net benefit) from the interaction of an individual with genotype j interating with a member of the other species with genotype k. Payout is determined by host and symbiont genotype and by the possible benefit (B_i) and the cost (C_i) of interaction. We assume that $B_i > C_i$, which restricts our analysis to conditions under which both partners can potentially receive a positive payout from the interaction.

Analytic models

We first derive analytic models of each of the three forms of mutualism, which consider the coevolution of hosts and symbionts and track allele frequencies in both species within a single population. Full details of model derivations and evaluation are available as Mathematica notebooks provided at github.com/jbyoder/mutualism-sanctions-recognition.

Host sanctions

First, consider a model of host sanctions against non-cooperative symbionts, in which symbiont cooperation and hosts' ability to sanction are each determined by a single

Table 1: Host and symbiont payouts under the model of host sanctions.

	Host	
Symbiont	H	h
	Host payout	
M	$B_H - C_H$	$B_H - C_H$
m	$(1-\omega)C_H$	$-C_H$
	Symbiont payout	
M	$B_S - C_S$	$B_S - C_S$
m	$(1-\omega)B_S$	B_S

biallelic locus. Symbionts with the *M* allele at a *cooperation* locus cooperate; symbionts with the *m* allele do not. Cooperative symbionts pay a cost of symbiosis, C_S, and receive a benefit, B_S, while non-cooperative symbionts receive the benefit but pay no cost. A host interacting with a cooperative symbiont pays a cost of hosting symbionts, C_H, and receives a benefit of symbiosis, B_H. Hosts interacting with non-cooperative symbionts receive no benefit, but pay the cost unless they are able to sanction.

Hosts with the *H* allele at a *sanctions* locus are able to stop interaction with a non-cooperating symbiont with effectiveness ω; hosts with the *h* allele are not able to do so. The term ω determines the degree to which sanctioning hosts are able to avoid paying the costs of hosting non-cooperating symbionts and deny them the benefit of symbiosis. If $\omega = 1$, sanctioning hosts suffer no cost of hosting non-cooperators and the non-cooperators receive no benefit; if $\omega = 0$, sanctions have no effect, so that hosts pay the full cost of symbiosis and non-cooperators receive the full benefit (Axelrod and Hamilton 1981; Ohtsuki 2010). We do not include a separate term for a cost paid by hosts when they apply sanctions, but a cost is implicit if sanctions are less than fully effective ($\omega < 1$) and there is a non-zero cost of hosting symbionts ($C_H > 0$). This parallels empirical systems in which sanctions cut off interaction after an initial investment, such as legumes that initiate nodulation with low-quality rhizobia only to reduce investment in less-productive nodules (Kiers et al. 2006), or yuccas and figs that invest in flowers, but abort them if they are too badly damaged by seed-feeding pollinators (Pellmyr and Huth 1994; Jandér and Herre 2010).

As noted above, host and symbiont fitness are equal to $1 + P_{jk}$, where P_{jk} is the payout from an individual with genotype j interacting with a member of the other species with genotype k, as determined by those genotypes, by the possible benefit (B_i) and the cost (C_i) of interaction to each species i, and by the effectiveness of sanctions ω (Table 1).

We can then derive the per-generation change in the frequency of the host's H allele:

$$\Delta p_H = p_H(1-p_H)\frac{\omega(1-p_M)C_H}{1-p_M B_H - [1-\omega p_H(1-p_M)]C_H} \quad (1)$$

And the symbiont's M allele:

$$\Delta p_M = p_M(1-p_M)\frac{\omega p_H B_S - C_S}{1+[1-\omega p_H(1-p_M)]B_S - p_M C_S} \quad (2)$$

Partner recognition

Next, consider a model of partner recognition, in which hosts only interact with symbionts expressing a signal compatible with the hosts's recognition genotype, and symbiont signals are determined by a locus independent of the locus that determines whether symbionts cooperate. This parallels the genetics of host-symbiont compatibility in plants' mutualisms with rhizobia and mycorrhizae (Yang et al. 2010; Young et al. 2011; Epstein et al. 2012), and vertebrate immune system recognition and tolerance of beneficial gut microbes (Pflughoeft and Versalovic 2012), and it is essentially a "matching alleles" infection genetics model of the type used to study host-parasite interactions (Agrawal and Lively 2002).

As in the sanctions model, symbionts cooperate if they have the M allele at the cooperation locus, and do not if they have the m allele. In this model, they also carry either an S allele or an s allele at a *signaling* locus. Hosts have no ability to sanction non-cooperating symbionts; instead they carry either an R or r allele at a *recognition* locus. Hosts with the R allele initiate symbiosis only with symbionts carrying the S signaling allele, hosts with the r allele initiate symbiosis only with symbionts carrying s. With no ability to sanction, hosts' payouts are determined solely by whether symbionts carrying compatible signaling alleles are also cooperative (Table 2).

An exact analytic examination of equilibria in this model is impractical. However, if we assume that the costs and benefits of the interaction are small (Nuismer et al. 2010; Yoder and Nuismer 2010), that the effects of the symbiont cooperation (M) and signaling (S) loci are therefore not strongly epistatic, and that there is free recombination between symbiont loci, then alleles at these loci should remain in quasi-linkage equilibrium (QLE) conditions (Barton and Turelli 1991; Kirkpatrick et al. 2002). With these assumptions, we can approximate change in the frequency of the host R allele as

Table 2: Host and symbiont payouts under the model of partner recognition.

	Host	
Symbiont	R	r
	Host payout	
MS	$B_H - C_H$	0
Ms	0	$B_H - C_H$
mS	$-C_H$	0
ms	0	$-C_H$
	Symbiont payout	
MS	$B_S - C_S$	0
Ms	0	$B_S - C_S$
mS	B_S	0
ms	0	B_S

$$\Delta p_R \approx p_R(1-p_R)(2p_S-1)(p_M B_H - C_H) \tag{3}$$

We can similarly approximate change in the frequency of the symbiont's M allele

$$\Delta p_M \approx p_M(1-p_M)\left[p_S - p_R(2p_S-1) - 1\right] C_S \tag{4}$$

And in the frequency of the S allele

$$\Delta p_S \approx p_S(1-p_S)(2p_R-1)(B_S - p_M C_S) \tag{5}$$

We can also calculate an approximate per-generation rate of change in LD between alleles at the two symbiont loci. However, under our QLE assumptions, LD does not contribute to the approximated change in host or symbiont allele frequencies, and the approximation for change in LD reveals that it will remain negligibly small. (See derivation in the "Recognition" Mathematica notebook at github.com/jbyoder/mutualism-sanctions-recognition.)

Sanctions with recognition

Finally, consider a model in which hosts have separate loci for symbiont recognition and sanctions, and symbionts have separate loci for signaling and cooperation. Hosts

Table 3: Host and symbiont payouts under the model of recognition with sanctions.

Symbiont	Host HR	Hr	hR	hr
	Host payout			
MS	$B_H - C_H$	0	$B_H - C_H$	0
Ms	0	$B_H - C_H$	0	$B_H - C_H$
mS	$-(1-\omega)C_H$	0	$-C_H$	0
ms	0	$-(1-\omega)C_H$	0	$-C_H$
	Symbiont payout			
MS	$B_S - C_S$	0	$B_S - C_S$	0
Ms	0	$B_S - C_S$	0	$B_S - C_S$
mS	$(1-\omega)B_S$	0	B_S	0
ms	0	$(1-\omega)B_S$	0	B_S

initiate symbiosis only with symbionts carrying a signaling allele compatible with the hosts' genotype at the recognition locus (i.e., S with R or s with r), as in the partner recognition model. However, hosts are also able to sanction if they carry the H allele at the sanctioning locus, as in the host sanctions model.

As in the host recognition model, to develop a tractable model we assume that the costs and benefits of interaction are small, that there is no epistasis, and that there is free recombination between loci. The payout values for each possible combination of host and symbiont genotypes (Table 3) then lead to the following approximations of change in the allele frequency at each locus in each species. For the host, these are

$$\Delta p_H \approx p_H(1-p_H)(1-p_M)\left[1-p_S-p_R(2p_S-1)\right]\omega C_H \tag{6}$$

$$\Delta p_R \approx p_R(1-p_R)(2p_S-1)\left[p_M B_H - \omega p_H(1-p_M)C_H - C_H\right] \tag{7}$$

And, for the symbiont

$$\Delta p_M \approx p_M(1-p_M)\left[1-p_S-p_R(1-2p_S)\right](\omega p_H B_S - C_S) \tag{8}$$

$$\Delta p_S \approx p_S(1-p_S)(1-2p_R)\left\{\left[1-\omega p_H(1-p_M)\right]B_S - p_M C_S\right\} \tag{9}$$

As in the host-symbiont recognition model, the approximations for change in allele frequencies do not include terms for LD between host loci or between symbiont loci, meaning that LD does not affect the approximated change in allele frequencies for either species. As in the previous model of partner recognition alone, the approximations for change in LD in both species indicate that LD will remain negligible. (See derivation in the "Sanctions with recognition" Mathematica notebook at github.com/jbyoder/mutualism-sanctions-recognition.)

Individual-based simulations

The approximations made to derive the analytic results may limit these models' generality, and evaluation of equilibrium conditions provides a limited perspective given that few real biological communities exist at evolutionary equilibrium (Thompson 2013). Moreover, modeling dynamics in a single panmictic population misses the potential for divergence among geographically structured populations, which can be an important mechanism for diversification in coevolutionary systems (Nuismer et al. 1999; Thompson 2005; Yoder and Nuismer 2010; Thompson 2013). To account for a broader range of parameter space such as stronger fitness effects of mutualism, to evaluate results at non-equilibrium conditions, and to examine the effects of geographic population structure and diversification, we constructed an individual-based simulation of coevolution between hosts and symbionts in a metapopulation of sites linked by migration. (Parameters are given in Table 4 and provided, with simulation scripts, at github.com/jbyoder/mutualism-sanctions-recognition.)

The simulation follows the change in allele frequencies in 50 populations of K_i haploid individuals of each species i, linked by migration at a rate of m_i. We chose parameters to ensure that the interaction would be commensal or mutualistic (all $B_i \geq C_i$); and that symbionts would usually have larger population sizes than hosts and experience greater benefits from symbiosis. These asymmetries are seen in many mutualistic symbioses. The simulation starts by randomly creating individuals' genotypes of one or two loci (depending on the model simulated) based on starting allele frequencies drawn from an approximation of the allele frequency spectrum for a standard neutral coalescent model at equilibrium (Ganapathy and Uyenoyama 2009). After creation of the starting populations, the simulation proceeds through a generational cycle of migration among populations, interaction between hosts and symbionts in each population, and finally mating within populations with mating success determined by outcome of the host-symbiont interactions.

Migration. A proportion m_i of the individuals in each population are selected at random

Table 4: Parameter values for the individual-based simulations.

Parameter[1]	Host	Symbiont
For metapopulation structure		
K, per-site population size	$U(20, 200)$	$U(200, 2000)$
m, among-site migration rate	$U(0, 0.05)$	$U(0, 0.05)$
For interaction payouts		
C, cost of symbiosis	$U(0.01, 0.1)$	$U(0.001, 0.1)$
B, benefit of symbiosis	$C_H + U(0.01, 0.1)$	$C_S \times U(0.01, 1)$
ω, effectiveness of sanctions	$U(0.01, 1)$	$U(0.01, 1)$
For genetics		
r, recombination rate	$U(0, 0.5)$	$U(0, 0.5)$
μ, mutation rate	10^{-6}	10^{-6}

[1] Parameters are either point values, or drawn from a uniform distribution with range $U(min, max)$.

to join a global migrant pool, which are then distributed at random back among the populations.

Coevolutionary selection. Within each population, hosts interact with randomly-drawn symbionts. All hosts interact with symbionts, while symbionts that are not drawn for interaction are lost from the population. Each individual's payout from the interaction is then determined by its genotype and the genotype of its host or symbiont, following one of the models outlined above. Finally, fitness is calculated for each individual as the payout of the interaction plus a value drawn at random from a normal distribution with mean = 1 and standard deviation = 0.1. These fitness values are then used to determine the probability of reproduction in the next step.

Mating. Mating occurs between pairs of hermaphroditic individuals of each species. Pairs of individuals are drawn at random from the same population, with replacement, and with the probability of being drawn scaled by each individual's fitness value from the prior step. Each mating produces one offspring, with genotypes at each locus drawn from the parental genotypes. In two-locus species, recombination between loci occurs with probability r_i, and mutation from one allele to the alternate allele occurs with probability μ_i for each locus. Mating continues until K_i offspring are created, at which time the offspring replace their parents to begin the next generation.

We ran 500 simulations for each of the three models, and 500 simulations that had no interaction phase, which provide a neutral expectation for evolution in the absence of the

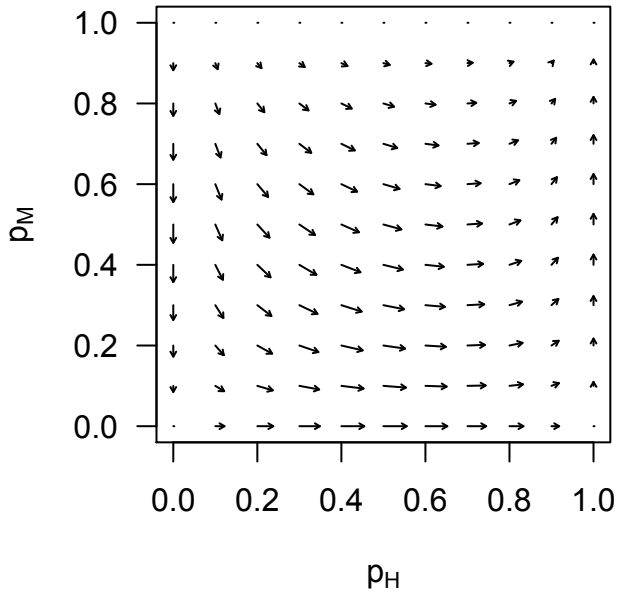

Figure 1: Dynamics of the host sanctions model. Vector-field plot indicating magnitude and dirction of change in the frequency of host sanctions (p_H) and symbiont cooperation (p_M) alleles at given starting frequencies, with $C_H = C_S = 0.25$, $B_H = B_S = 0.5$, and $\omega = 0.75$.

mutualism. We summarized simulation results after 1,000 generations, well past the time at which among-site variation in allele frequencies stabilized in all simulations.

Results

Analytic models

We solved for equilibria in each of the three analytic models (sanctions only, recognition only, and sanctions with recognition) to identify conditions that maintain variation in host or symbiont loci, and that maintain mutualism (i.e., the frequency of symbiont cooperation, $p_M > 0$. Full details of these analyses are given in Mathematica notebooks, available at github.com/jbyoder/mutualism-sanctions-recognition.

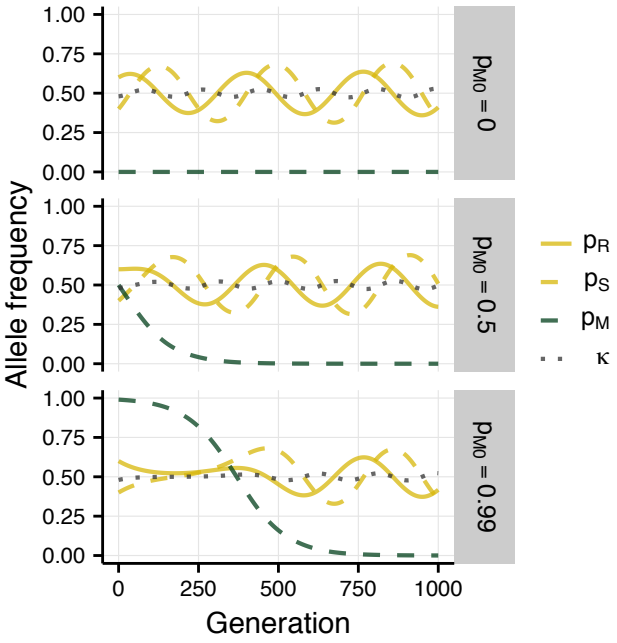

Figure 2: Dynamics of partner recognition. Allele frequencies over time at host recognition (solid gold lines) and symbiont signaling (dashed gold lines) loci, allele frequency at the symbiont cooperation locus (dashed green lines), and host-symbiont compatibility, κ, (dotted gray lines), with intial frequency of the symbiont cooperation allele $p_{M0} = 0$, $p_{M0} = 0.5$, or $p_{M0} = 0.99$, and with $C_H = C_S = 0.025$ and $B_H = B_S = 0.05$.

Sanctions

The sanctions model has locally unstable equilibria when $p_M = 0$ and p_H is equal to either 1 or 0; and when $p_M = 1$ at any value of p_H. There are no stable equilibria that maintain variation in either sanctions or cooperation (Figure 1). Although there are no equilibria that maintain variation at either sanctions or cooperation loci, the rate of change in the frequencies of sanctioning and cooperation alleles is very low whenever p_M or p_H are near 1, meaning that it may take considerable time for these alleles to become fixed.

Partner recognition

In the partner recognition model there are equilibria that maintain variation, with cyclical dynamics, at recognition and signaling loci ($pR = pS = 0.5$), but only when the cooperation allele (M) is fixed or entirely absent (Figure 2). There are also equilibria that maintain variation in cooperation (i.e., M is at intermediate frequency), but only when the recognition and signaling loci are fixed for incompatible alleles. In other words, the system can

maintain variation in recognition, but only when that recognition has no consequences for fitness; and it can maintain variation for cooperation, but only when symbiosis is never initiated. Under these conditions variation maintained at mutualism-related loci is effectively neutral, and would be lost via drift in finite populations.

Variation at signaling loci is maintained by inverse frequency dependent selection when the cooperation allele is lost from the population ($p_M = 0$). However, this is not surprising given that when $M = 0$ the system is effectively a host-parasite system, which have been repeatedly shown to maintain variation with a 2-allele system determining host recognition (Dieckmann et al. 1995; Agrawal and Lively 2002). We can examine these dynamics in terms of host-symbiont *compatibility*, or the probability that a randomly-drawn host and symbiont will carry compatible recognition and signaling alleles, defined as $\kappa = (p_R * p_S) + (1 - p_R)(1 - p_S)$. This probability remains close to 0.5 even as recognition and signaling allele frequencies cycle (Figure 2).

Perhaps the most important feature of the model of partner recognition model the is that its approximation of change in the frequency of the symbiont cooperation allele, Δp_M, is ≤ 0 for all reasonable parameter values (Equation 4; see also the "recognition" Mathematica notebook at github.com/jbyoder/mutualism-sanctions-recognition). That is, there is no condition under which a cooperative symbiont allele (M) will increase in frequency. This means that the system can only remain a mutualism ($p_M > 0$) if the cooperation allele is already present in the population; and that partner recognition alone cannot select for greater frequency of cooperation, from any starting frequency (Figure 2).

Sanctions with recognition

The model of partner recognition with host sanctions has equilibria that maintain variation at both host recognition and symbiont signaling loci. Still, the only locally stable equilibria at which hosts or symbionts maintain variation in sanctioning or cooperation also have hosts and symbionts fixed for incompatible recognition/signaling alleles — conditions ensuring that symbiosis is never initiated. Non-equilibrium conditions exist that can maintain variatin in host sanctions or symbiont cooperation without complete loss of host-symbiont compatibility (Figure 3), though these depend on starting allele frequencies, the cost and benefit of mutualism to each species, and particularly the effectiveness of sanctions.

If sanctions are not very effective (i.e., ω is low) then symbiont cooperation may be lost even as host sanctions become fixed; loss of cooperative symbionts leads to inverse frequency-dependent cycling at the host recognition and symbiont signaling loci, as seen in a host-pathogen model (Figure 3, upper panels). With more effective sanctions,

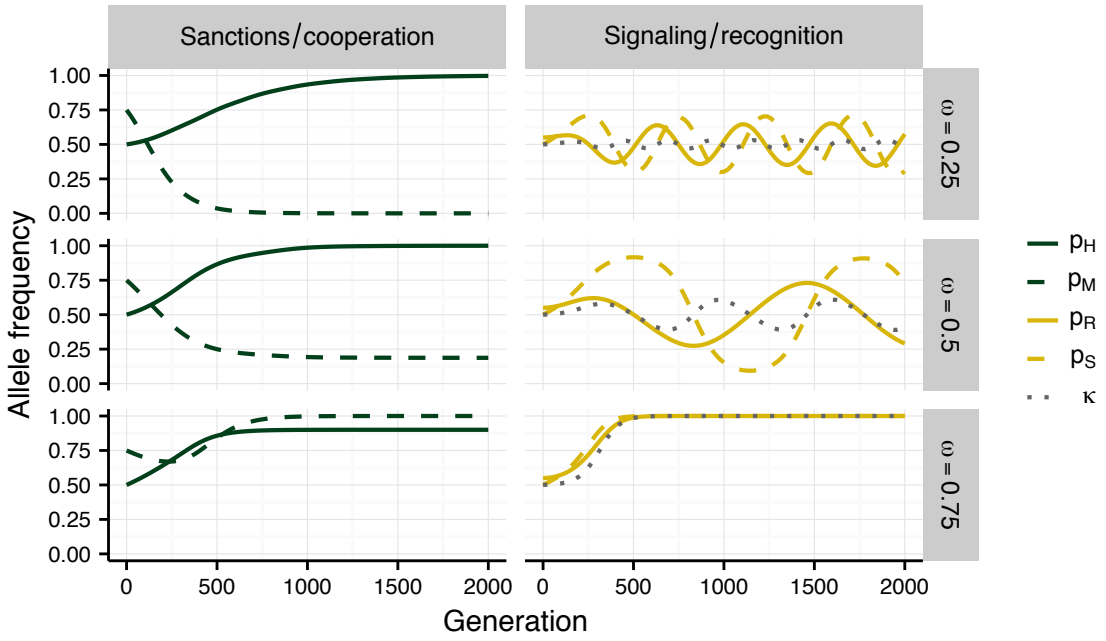

Figure 3: Dynamics of host sanctions with partner recognition. Allele frequencies over time at host (solid lines) and symbiont (dashed lines) sanctions/cooperation loci (left, green) or recognition/signaling loci (right, gold), when the effectivness of sanctions $\omega = 0.25$ (top), 0.5 (middle), or 0.75 (bottom). In plots of allele frequency at the signaling/recognition loci (right), a dotted black line indicates host-symbiont compatibility, κ. For all scenarios, the initial frequency of the host sanctions allele $p_H = 0.5$, host recognition allele $p_R = 0.55$, symbiont cooperation $p_M = 0.75$, symbiont signaling $p_S = 0.5$, $C_H = C_S = 0.025$ and $B_H = B_S = 0.05$.

symbiont cooperation can be maintained at low frequency, while inverse frequency-dependent cycles at the signaling and recognition loci occur with a longer period — reflecting the fact the symbiosis is, on average, costly to hosts so long as fewer than half of symbionts are cooperative (Figure 3, middle panels).

When sanctions are sufficiently effective, variation can be maintained at the host sanctions locus once symbiont cooperation is fixed and the recognition/signaling loci become fixed for compatible alleles (Figure 3, lower panels). This parallels results from the model of host sanctions alone (Figure 1), in which fixation of symbiont cooperation reduces Δp_H to zero (Equation 1). Indeed, when the signaling and recognition loci are fixed for compatible alleles ($\kappa = 1$), the model of sanctions with recognition behaves like the model of sanctions alone.

Meanwhile, the relative fitness of different symbiont signaling alleles depends not only

on the frequency of host recognition alleles, but also on the frequency of symbiont cooperation, the frequency of sanctioning hosts, and the degree to which sanctions reduce the fitness of non-cooperating symbionts. This is apparent from the approximate expression for change in the frequency of the symbiont signaling allele, Δp_S, which has an unstable equilibrium when $p_H = 1$ and $C_S/B_S = 1 - \omega(1 - p_M)$ (Equation 11).

Host-symbiont compatibility, κ, increases most rapidly when most symbionts are cooperative, when sanctioning hosts are more common, and when hosts have near-maximum variation at the recognition locus (p_R is near 0.5) but symbionts are nearly fixed for one signaling allele (Figure 4, dark-shaded regions). It decreases most rapidly when hosts are mostly unable to sanction, cooperative symbionts are at lower frequency ($p_M < 0.5$), and hosts mostly carry a recognition allele compatible with the more-common symbiont allele (Figure 4, light-shaded regions).

Individual-based simulations

Sanctions

After 1,000 generations, individual-based simulations of the sanctions model ended with the host sanctioning allele at significantly higher global frequency than expected from neutral simulations (Figure 5A; $p < 1 \times 10^{-6}$, t-test on arcsine-transformed values). This is consistent with the predictions of the analytic model (Figure 1). Still, the sanctioning allele achieved fixation in just 21% of simulations, and there was considerable overlap in range of frequencies seen for the sanctioning allele and that for neutral alleles. Simulations in which sanctions fixed had significantly higher costs of symbiosis for the host than simulations in which they did not (mean C_H of 0.06 in simulations with $p_H = 1$, 0.05 otherwise; t-test $p < 0.001$); and also had higher benefits, though this difference was not statistically significant (mean B_H of 0.12 when $p_H = 1$, 0.11 otherswise; t-test $p = 0.09$).

In the same simulations, the symbiont cooperation allele rose to significantly higher global frequency than expected from the neutral simulations (t-test $p < 1 \times 10^{-6}$), and it became fixed in 59% of simulations of sanctions, compared to less than 2% of neutral simulations that achieved fixation — consistent with the predictions of the analytic model (Figure 1). However, the cooperation allele was also lost in 21% of simulations. This is partly explained by the payout of the symbiosis; simulations in which the cooperation allele became fixed had significantly lower costs of symbiosis for the symbiont than those in which cooperation did not become fixed (mean C_S of 0.048 in simulations with $p_M = 1$, 0.058 otherwise; t-test $p < 1 \times 10^{-4}$), and had significantly greater benefits (mean B_S of 0.59 when $p_M = 1$, 0.48 otherwise; t-test $p < 1 \times 10^{-4}$). Greater frequency of

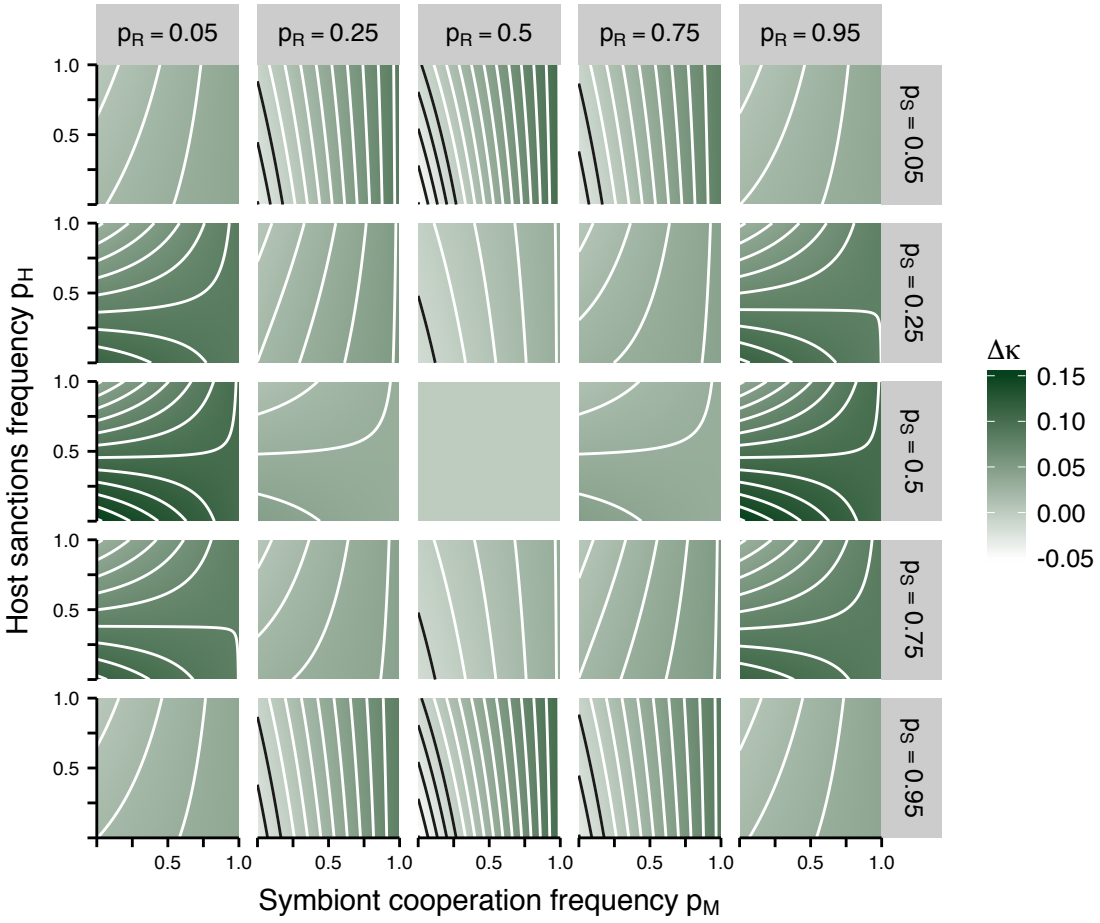

Figure 4: Per-generation rate of change in κ, host-symbiont compatibility, for different starting frequencies of the symbiont cooperation allele p_M and host sanctions allele p_H, given different values of the host recognition allele p_R and symbiont signaling allele p_S. Darker shading indicates greater values of $\Delta\kappa$; contour lines are at intervals of 0.01, with white lines indicating values of $\Delta\kappa > 0$. For all panels $\omega = 0.75$, $B_S = B_H = 0.75$, and $C_S = C_H = 0.25$.

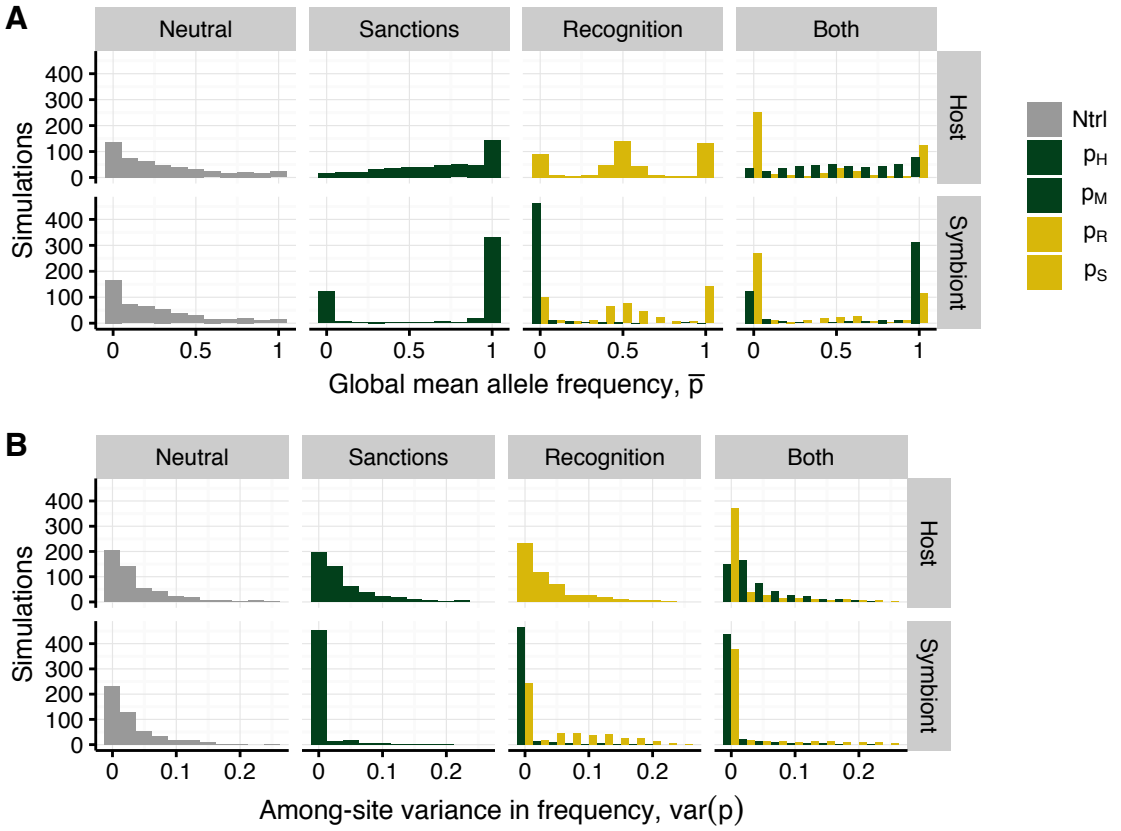

Figure 5: Outcomes of individual-based simulations for each of the three genetic models of symbiosis in a metapopulation of sites linked by migration. Distributions of (A) global mean of within-population allele frequencies and (B) among-site variation in local allele frequency after 1,000 generations of coevolution in 500 replicate simulations run with parameters given in Table 4.

symbiont cooperation was also associated with greater frequency of the sanctioning allele; simulations in which symbiont cooperation was lost had significantly lower frequency of the host sanctioning allele (mean p_H of 0.65 when $p_M = 0$, 0.76 when $p_M = 1$; $p < 1 \times 10^{-5}$ in a t-test on arcsin-transformed values).

To examine geographic differentiation among sites, we calculated the among-site variance in allele frequencies for each replicate simulation (Figure 5B). In simulations of the sanctions model, among-site variance in the frequency of the host sanctions allele was very similar to that seen for neutral simulations. The symbiont cooperation locus, however, had much lower among-site variation than seen in the neutral simulations, with 80% of simulations having $var(p_M) = 0$ (this was seen in 20% of neutral simulations). This reduced geographic variation reflects the high proportion of simulations in which

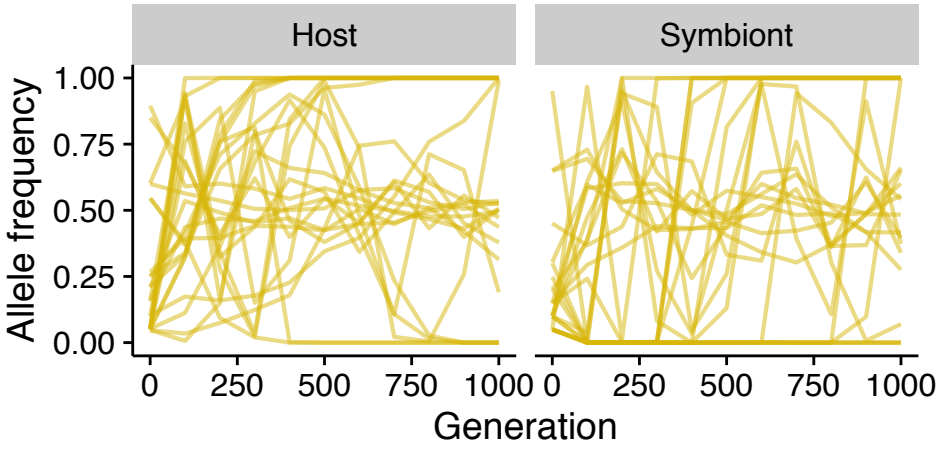

Figure 6: Cyclical dynamics in individual-based simulations of recognition. Plots of allele frequency over time at the host recognition locus (p_R; left) and symbiont signaling locus (p_S; right), in a sample of 30 replicate simulations.

symbiont cooperation became globally fixed (Figure 5A).

Partner recognition

In simulations of recognition alone, the symbiont cooperation allele was lost in the vast majority (79%) of simulations, as predicted by the analytic model (Figure 2; Figure 5A). Consistent with the cyclical dynamics predicted by the analytic model host recognition alleles were more likely to be at mid-frequencies than seen in neutral loci ($0.4 < p_R < 0.6$ in 42% of simulations, versus 12% for neutral host loci), and the same was true for symbiont signaling alleles ($0.4 < p_S < 0.6$ in 28%, versus 10% for neutral symbiont loci). Signaling and recognition alleles were also often fixed or lost, but could resume cyclical dynamics when reintroduced by mutation (Figure 6). As a result of these cyclical dynamics, the correlation between allele frequencies at the host recognition locus and the symbiont signaling locus varied dramatically over time, from strongly negative (at generation 300, Spearman's $\rho = -0.17$, $p < 1 \times 10^{-4}$), to strongly positive (at generation 1,000, $\rho = 0.23$, $p < 1 \times 10^{-6}$).

Patterns of among-site variation at the symbiont signaling and host recognition loci were broadly similar to those seen for neutral loci (Figure 5B). However, the symbiont cooperation locus had much lower among-site variation than either the signaling locus or the neutral expectation ($var(p_M) = 0$ in 79% of simulations, $var(p_S) = 0$ in 42%; and 22% of neutral simulations had $var(p) = 0$), a result of the high frequency with which

the cooperation allele was lost.

Sanctions with recognition

At generation 1,000, simulations of sanctions with recognition had, on average, somewhat lower frequency of sanctioning hosts than seen in simulations of sanctions alone (t-test on arcsin-transformed data, $p < 1 \times 10^{-6}$), and were less likely to have sanctions fixed ($p_H = 1$ in 11% of simulations of sanctions with recognition). As in simulations of sanctions alone, simulations of sanctions with recognition in which the sanctioning allele became fixed had significantly higher costs of symbiosis for hosts (mean C_H of 0.07 for simulations with $p_H = 1$, 0.05 otherwise; t-test $p < 1 \times 10^{-6}$), and also had somewhat higher benefits of symbiosis (mean B_H of 0.12 when $p_H = 1$, 0.11 otherwise; t-test $p = 0.01$).

The fate of the symbiont cooperation alleles in the sanctions with recognition model was also similar to results from the simulations of sanctions alone, with most simulations having the cooperation allele going to fixation (56% of simulations) or lost (21%). Also as in the simulations of sanctions alone, the fixation of the symbiont cooperation allele was associated with significantly lower costs of symbiosis (mean C_S of 0.046 when $p_M = 1$, 0.053 otherwise; t-test $p = 0.001$) and significantly greater benefits of symbiosis (mean B_S of 0.62 when $p_M = 1$, 0.55 otherwise; t-test $p < 1 \times 10{-}4$). However, contrary to what is seen in the simulations of sanctions alone, simulations of sanctions with recognition in which symbiont cooperation was lost did not have significantly lower frequency of sanctioning hosts than simulations with $p_M > 0$ (mean p_H of 0.55 when $p_M = 0$, 0.56 otherwise; $p = 0.44$ in a t-test on arcsin-transformed values).

The frequency of host recognition alleles was strongly and positively correlated with the frequency of symbiont signaling alleles at all timepoints (at generation 1,000, $\rho = 0.80$, $p < 1 \times 10^{-6}$), and recognition and signaling alleles were at intermediate frequency in fewer simulations of sanctions with recognition ($0.4 < p_R < 0.6$ in 12% of simulations; $0.4 < p_S < 0.6$ in just 9%) compared to simulations of recognition alone. This is consistent with hosts and symbionts converging on compatible signaling and recognition alleles, and maintaining them at high frequency — something also seen in the analytical model when ω was high. Indeed, in simulations of sanctions with recognition, host-symbiont compatibility (κ) was generally much higher than in the model of recognition alone (Figure 7, $p < 1 \times 10^{-6}$ in a t-test on arcsin-transformed values).

The two mechanisms (i.e., sanctions/cooperation and recognition/signaling) evolved in response to each other as predicted by the analytic model (Figures 3 and 4). In simulations of sanctions with recognition, host-symbiont compatibility was strongly and

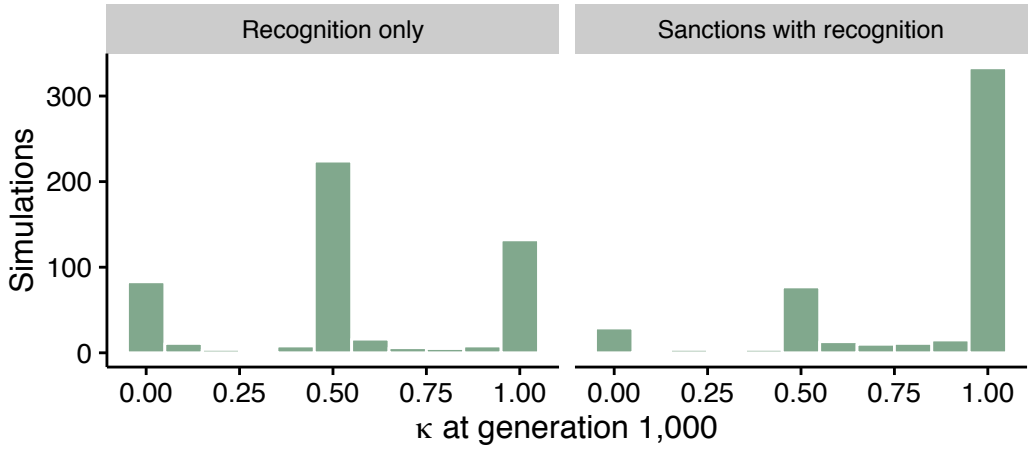

Figure 7: Host-symbiont compatibilty, κ, after 1,000 generations of coevolution in simulations of recognition alone (left) or sanctions with recognition (right).

positively correlated with the frequency of sanctioning hosts, p_H, at early time points in the simulation, but this correlation decreased over time, possibly due to fixation of the sanctioning allele in many replicates (tests on arcsine-transformed values; at generation 100, Spearman's $\rho = 0.25$, $p < 1 \times 10^{-6}$; at generation 500, $\rho = 0.17$, $p = 0.001$; at generation 1000, $\rho = 0.05$, $p = 0.30$).

Notably, simulations of sanctions with recognition maintained significantly more among-site variation at the sanctions locus than simulations of sanctions alone (t-test on arcsin-transformed values; $p = 0.005$), and maintained much less among-site variation at the host recognition locus than simulations of recognition alone (t-test $p < 1 \times 10^{-6}$), largely due to the higher rate of fixation in the simulations of sanctions alone. Simulations of sanctions alone and sanctions with recognition both resulted in very low among-site variation at the symbiont cooperation locus, with $var(p_M) = 0$ in 76% of simulations and 70% of simulations, respectively.

Discussion

Variation in the quality of mutualistic partners is widely observed in natural systems, (Pellmyr and Huth 1994; Herre and West 1997; Holland et al. 1999; Simms and Taylor 2002; Ness et al. 2006; Heath and Tiffin 2009; Hoeksema 2010; reviewed by Heath and Stinchcombe 2014), in spite of the fact that mechanisms that stabilize mutualisms against cheating should remove such variation over time (Axelrod and Hamilton 1981;

Doebeli and Knowlton 1998; West et al. 2002b; Kopp and Gavrilets 2006; Yoder and Nuismer 2010). Genetic variation may be maintained in the face of selection by mutation-selection balance (Foster et al. 2006) or by drift and migration among spatially structured populations (Thompson et al. 2013; Heath and Stinchcombe 2014), but none of the forms of coevolutionary selection typically expected between mutualists can contribute to maintaining variation.

Our model of symbiotic mutualism in which hosts recognize symbiont signals as well as sanctioning non-cooperative symbionts shows how coevolutionary selection between mutualists can both stabilize mutualism and maintain variation in its outcomes. Neither sanctions or partner recognition alone can maintain mutualism and variation in mutualism outcomes. However, in a model that includes both mechanisms, variation can be maintained either because hosts and symbionts vary in sanctioning ability and cooperation, or because they vary in their signaling/recognition compatibility (Figure 3). Our individual-based simulations corroborate the prediction from the analytic model that the sanctions and recognition systems interact by altering the coevolutionary conditions each genetic system faces (Figures 5, 7).

Sanctions versus recognition, solo and in concert

Similar to previous models of mutualism (Trivers 1971; Axelrod and Hamilton 1981; Bull and Rice 1991; West et al. 2002a; West et al. 2002b; Foster and Wenseleers 2006), we found that host sanctions maintain cooperative symbionts at high frequency (Figures 1, 5). The advantage of non-cooperation may cause the frequency of cooperative symbionts to decrease, but lower frequency of cooperation increases the relative fitness of sanctioning hosts — and once sanctions are sufficiently common, the frequency of cooperative symbionts increases to fixation (Figure 1).

On the other hand, when partner signals and cooperation are determined by unlinked loci, partner recognition alone is unable to select for greater frequency of cooperative symbionts (Equation 4), and when cooperative symbionts are lost selection favors hosts compatible with whichever symbiont signaling allele is less common (Figure 2). Because hosts gain no benefit from the symbiosis, this situation is effectively the loss of mutualism, and it creates coevolutionary cycles in the frequency of signaling and recognition alleles (Figures 2, 6), similar to what is seen in host-pathogen systems (Dieckmann et al. 1995; Agrawal and Lively 2002; M'Gonigle and Otto 2011).

In contrast to these simpler systems, when hosts both selectively initiate symbiosis based on recognition of symbiont signals and are also able to sanction non-cooperative

symbionts, the system can maintain variation in symbiont cooperation and at recognition and signaling loci (Figure 3, middle panels). However, this occurs when sanctions have intermediate effectiveness; if sanctions are less effective, the same starting conditions and interaction payout result in loss of cooperation (Figure 3, top panels); whereas if sanctions are stronger, symbiont cooperation becomes fixed and recognition and signaling loci fix for compatible alleles (Figure 3, bottom panels). Once cooperation is fixed, the host sanctioning allele is effectively neutral, and variation at that locus is expected to be lost to drift.

In our simulations, this interaction between sanctions and recognition leads to somewhat less frequent fixation of the host sanctioning allele, and more among-site variation in its frequency (Figure 5A, 5B), even as symbiont cooperation evolves to a frequency close to that seen in simulations of sanctions alone (Figure 5A). All of these outcomes are connected to the fact that when hosts can sanction non-cooperative symbionts there is less selective advantage to avoiding symbiosis, and hosts and symbionts converge on compatible recognition and signaling alleles (Figure 7).

This result is similar to those from models of mutualism based on economic contract theory, which propose that sanctioning is often best understood not as a specific adapation to minimize the cost of interaction with non-cooperative partners (Weyl et al. 2010; Archetti et al. 2011a; Archetti et al. 2011b), but as pre-existing characteristics of the mutualists that provide partner fidelity feedback by positively responding to cooperative symbionts (Archetti et al. 2011a). Under this thinking, floral abortion in response to pollinator overexploitation in brood pollination mutualisms is a repurposing of plants' response to floral damage; legumes' reduced allocation to underproductive root nodules may arise from adaptations for root growth in soil with heterogenous nutrient content (Pellmyr and Huth 1994; Kiers et al. 2006; Weyl et al. 2010). In our model, interaction with symbionts of varying quality favors higher frequency, and often fixation, of sanctioning hosts (Figures 3, 5A). In turn, high frequency of sanctioning hosts relaxes selection for host recognition alleles that prevent symbiosis, leading to higher host-symbiont compatibility (Figures 4, 7). This recapitulates the result of Archetti *et al.*, (2011a) that hosts offering the right "terms" to symbionts need not screen for cooperative symbionts prior to initiating the interaction. (In our model, the "terms" offered to symbionts would be that hosts will not sanction if symbionts cooperate.)

Although few "classic" mutualisms involve two haploid partners, as in our models, we do not believe that relaxing this simplifying assumption would change our conclusions. M'Gonigle and Otto (2011) modeled the effect of varying host and symbiont ploidy in a matching-alleles model similar to the signaling-recognition system we consider, and found that diploidy made hosts better able to recognize and resist both haploid and

diploid parasites. This suggests that a diploid version of our partner recognition model would see hosts better able to evade symbiosis when cooperative symbionts are rare, but such evasion does not select for more cooperative symbionts in our haploid model (Figure 2). A diploid model of host sanctions, meanwhile, could allow more continuous variation in the effectiveness of sanctions and the degree of symbiont cooperation, but the fundamental dynamic of sanctions selecting for more cooperative symbionts should remain (Figure 1, 3).

Cooperation and communication in mutualism

Our results suggest that empirical study of mutualism should anticipate that multiple genetic mechanisms, which may experience very different forms of coevolutionary selection, contribute to the evolution cooperating species. This can be achieved by experimental designs that explicitly separate the exchange of benefits from the initiation of a mutualism (e.g., Regus et al. 2014; Althoff 2016; Powell and Doyle 2016), and in population genetic studies that test for different signals of selection at loci with different roles in mutualism (e.g., Paape et al. 2013; Bonhomme et al. 2015; Yoder 2016).

There is evidence in many classic coevolved mutualisms for communication between partners that is based on traits separate from the rewards provided to (or withheld from) those mutualists (Svensson et al. 2005; Edwards et al. 2006b; Okamoto et al. 2007; Soler et al. 2011; Svensson et al. 2016). The system in which the relationship between partner signals and response to partner performance is best understood may be the symbiotic mutualism of legumes and nitrogen-fixing rhizobial bacteria. Legumes sanction ineffective rhizobia (Kiers et al. 2003; Kiers et al. 2006; Regus et al. 2014), but they also respond to molecular signals from rhizobia as they establish symbiosis (Triplett and Sadowsky 1992; Oldroyd et al. 2011). At the level of quantitative phenotypes, host-rhizobium compatibility is at least partly independent of variation in mutualism outcomes (Triplett and Sadowsky 1992; Bena2005a; Heath and Tiffin 2009; Grillo et al. 2016; Powell and Doyle 2016).

Members of legume gene families associated with pathogen recognition are also implicated in legume-rhizobium compatibility (Yang et al. 2010; Young et al. 2011), and some legume genes with roles in the symbiosis show elevated nucleotide diversity and geographic differentiation consistent with frequency-dependent dynamics (Yoder 2016). However, rhizobial genes producing nodule initiation factors — signals recognized by hosts — have reduced diversity relative to the rest of the genome, consistent with selective sweeps or purifying selection (Bailly et al. 2006). Rhizobial type III effector genes, which may also be recognized by hosts, similarly show patterns of greater sequence conser-

vation than homologous genes in pathogenic bacteria (Kimbrel et al. 2013). Another complicating factor is that many rhizobia species have genes involved in signaling and nitrogen fixation physically linked in a "symbiosis island" (e.g., Sullivan and Ronson 1998; Laguerre et al. 2001; Parker 2012), which reduces the opportunity for separate coevolutionary dynamics related to signaling and cooperation. Still, rhizobial genes mediating host recognition can exhibit signs of negative frequency-dependent selection when they are not in close linkage with nitrogen fixation genes (Bailly et al. 2006), and genes involved in both signaling and nitrogen fixation show signs of elevated horizontal gene transfer (Bailly et al. 2006; e.g., Sun et al. 2006; Epstein et al. 2012; Parker 2012).

Sequence conservation at rhizobial signaling genes may also be explained by one of our key results, in which hosts' ability to sanction allows hosts and symbionts to converge on compatible alleles at recognition and signaling loci (Figures 3, 6). Once this convergence occurs, there should be strong selection against symbionts expressing signals incompatible with the more common host genotype. This hypothesis predicts that frequency dependent selection on rhizobial signaling loci and legume recognition loci should manifest when cooperative rhizobia are rare, or when legume sanctions are ineffective (Figure 3, top panel). This could be tested by comparing populations that vary in the frequency of cooperative rhizobia or sanctioning legume genotypes, or possibly by experimental evolution. There is some suggestion that selection of crop legume cultivars has reduced their capacity to screen out and sanction less-cooperative rhizobia (Kiers et al. 2007), and other human manipulations of legumes' environment may offer similar natural or inadvertent experiments in the form of selection acting on legume-rhizobium signaling.

Conclusions

Mutualistic interactions require communication between potential partners as well as cooperation once the interaction is underway. Previous theory of mutualism has not, however, explicitly included both of these systems of interaction. Our model of a symbiotic mutualism incorporating host recognition of symbiont signals alongside host sanctions against non-cooperative symbionts proves to better reflect the apparent contradictions of empirical systems, maintaining mutualism and variation in interaction outcomes under conditions where neither system, on its own, can do so.

Data archiving

Full derivation and analysis of our analytic models, and scripts for individual-based simulations, are online at github.com/jbyoder/mutualism-sanctions-recognition.

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