

LETTER

The cryptic role of biodiversity in the emergence of host–microbial mutualisms

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Abstract

The persistence of mutualisms in host-microbial – or holobiont – systems is difficult to explain because microbial mutualists, who bear the costs of providing benefits to their host, are always prone to being competitively displaced by non-mutualist ‘cheater’ species. This disruptive effect of competition is expected to be particularly strong when the benefits provided by the mutualists entail costs such as reduced competitive ability. Using a metacommunity model, we show that competition between multiple cheaters within the host’s microbiome, when combined with the spatial structure of host–microbial interactions, can have a constructive rather than a disruptive effect by allowing the emergence and maintenance of mutualistic microorganisms within the host. These results indicate that many of the microorganisms inhabiting a host’s microbiome, including those that would otherwise be considered opportunistic or even potential pathogens, play a cryptic yet critical role in promoting the health and persistence of the holobiont across spatial scales.

Keywords

Competition, holobiont, host microbiome, indirect interactions, metacommunity theory, microbial diversity, mutualisms, patch-dynamic.

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INTRODUCTION

Mutualisms involving cases where multi-species microbial guilds interact with one or more host partners are a ubiquitous feature of natural systems (Stanton 2003). The mutualistic interactions that characterise the relationship between host organisms and their microbial endosymbiont communities, in particular, have been extensively studied in a number of systems, including the microbial communities (dinoflagellates and bacteria) associated with corals (Knowlton & Rohwer 2003; Krediet *et al.* 2013), fungal and bacterial nutritional endosymbionts associated with plant root systems (reviewed in Denison 2000; Bever *et al.* 2009), as well as the gut microflora of insects (Ohkuma & Kudo 1996; Aksoy *et al.* 1997; Dillon & Dillon 2003) and humans (Bäckhed *et al.* 2005; Peterson *et al.* 2008; Round & Mazmanian 2009; Sekirov & Finlay 2009; Clemente *et al.* 2012). Because these mutualists provide key services such as nutrient subsidies and improved immunodefences, their persistence within the host microbiome is often critical to the health and normal functioning of their respective hosts.

However, the diversity of symbionts within these microbial communities poses a serious evolutionary problem because the presence of multiple microbial symbionts in a single host is likely to set the stage for competition, which could potentially threaten the stability and persistence of any mutualism within the host–microbial (holobiont) system. Indeed, symbiont diversity increases the likelihood that competition will lead to the replacement of mutualistic traits by less beneficial ones because mutualists are likely to be at a competitive disadvantage relative to ‘cheater’ strategies that bear none of the costs associated with providing resources to the host (Herre *et al.* 1999; Knowlton & Rohwer 2003; Stanton 2003).

Until now, the persistence of mutualists has been primarily explained as resulting from the host playing an active role in partner choice by selecting and rewarding beneficial microbes, or by excluding non-beneficial microbes via the deployment of sanctions (West *et al.* 2002; Hoeksema & Kummel 2003; Stanton 2003; Scheuring & Yu 2012; Schluter & Foster 2012). Other mechanisms have also been implicated in the stabilisation of mutualisms in holobiont systems. These include the vertical transmission of beneficial symbionts from host parent to offspring (Herre *et al.* 1999) and the targeted inhibition of cheaters via interference competition among species within the host microbiome (Massey *et al.* 2004; Scheuring & Yu 2012), a phenomenon that has been observed in the mucus layer of corals where various microbes help block pathogens by secreting antibiotics specific to non-beneficial microbes (Ritchie 2006; Mao-Jones *et al.* 2010; Kvennefors *et al.* 2011).

Although effective, vertical symbiont transmission is unlikely to provide a general explanation for the persistence of mutualists in nature because most host–microbial systems involve horizontal transmission of the symbiont (i.e., the symbionts are acquired anew each generation; Herre *et al.* 1999; Knowlton & Rohwer 2003). Hence, the stabilising effect of partner fidelity on mutualisms that is made possible by vertical transmission is unavailable in many key holobiont systems. Similarly, host selection and targeted inhibition by other microbes usually requires that a fine-tuned system for recognising cheaters already be in place. Such a system would require a relatively fixed species-recognition strategy capable of discriminating against parasites or cheaters *a priori* by specifically targeting cheaters before any serious investment in the mutualism has been made by the host. In the case of host selection, this problem of distinguishing between beneficial and non-beneficial microbes can potentially be overcome in

special cases where the endosymbiotic environment within hosts is characterised by a spatial or modular structure that can allow distinct microbial subpopulations to be targeted individually for rewards or sanctions, as in the root nodules of plants (Denison 2000; Hoeksema & Kummel 2003; Bever *et al.* 2009; Kiers *et al.* 2011).

In the absence of within-host spatial or modular structure, it becomes difficult to explain how host selection alone (as well as targeted inhibition through microbial interference competition) can allow mutualisms to evolve in the first place when naïve hosts are unable to distinguish between beneficial and deleterious microbes. This is particularly true when the dynamics of the host are much slower than that of the microbes. Indeed, under such conditions, deleterious cheaters are expected to rapidly exclude beneficial mutualists before the host is able to develop sanctions against cheaters.

Regardless of the specific mechanisms assumed to be involved in stabilising host–microbial mutualisms, all current theory starts from the assumption that general resource competition arising from diversity within the microbiome has a universally *disruptive* effect on the persistence of mutualistic microbes. What has not been considered, until now, is the possibility that resource competition may play a *constructive*

role in facilitating host–microbial mutualisms. Here, we show that when both the multi-species nature (Johnson *et al.* 2013) and spatial structure (Mihaljevic 2012) of host–microbial interactions are modelled, the presence of multiple competing cheater strategies can allow mutualisms to evolve and persist in the absence of vertical transmission, host selection and targeted interference competition.

MATERIALS AND METHODS

We develop a patch–dynamic metacommunity (Levins 1969) model to represent a generic host–microbial system consisting of a network of local microbial communities interconnected by dispersal (Leibold *et al.* 2004). Individual hosts, which serve as microbial habitat patches, are born at rate c and die at rate e (Fig. 1). The dynamics of the microbes are determined by both local competitive interactions within a host and by regional-scale colonisation-extinction dynamics.

There are three basic microbial species: a mutualistic symbiont x , a strongly competitive cheater z , and a weakly competitive cheater y . Here, ‘strongly’ and ‘weakly’ competitive refer to the strength of competitive pressure exerted by the cheater on the mutualist within the host. Within a host, the three

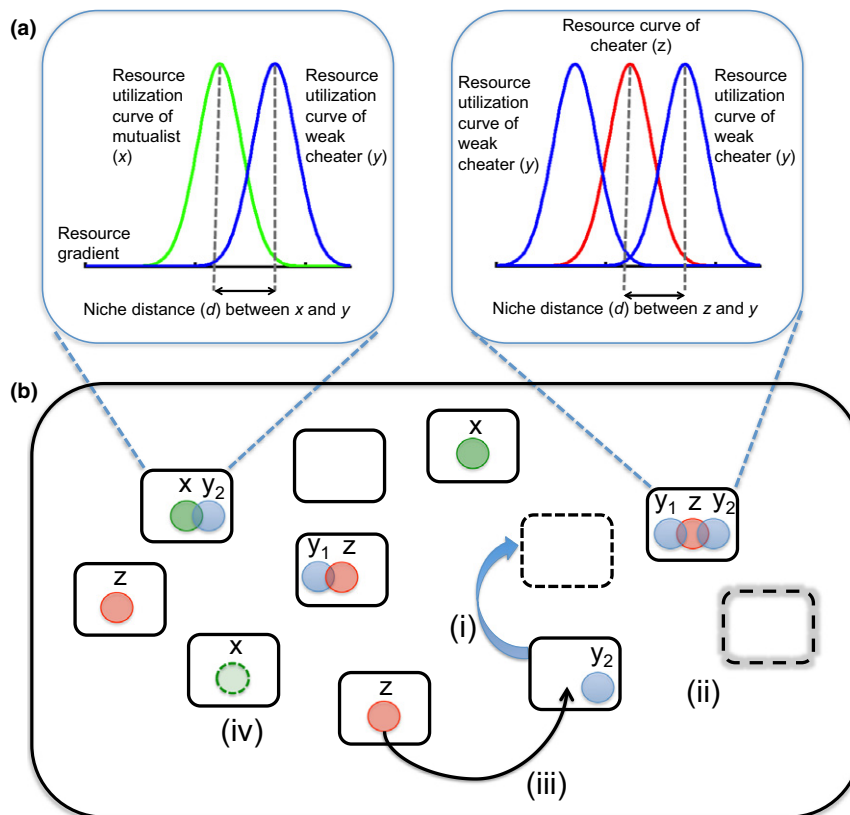


Figure 1 Diagram of the metacommunity model. (a) Local (within-host) resource competition between microbes arising from overlapping resource niches of x , z and y . (b) Metacommunity-scale dynamics arising via colonisation and extinction of microbial populations, and the growth and death of host organisms. Rectangles represent host individuals that serve as habitat patches for microbial communities, several examples of which are depicted. Microbial populations within communities are represented as follows: mutualist populations (green circles), strongly competitive cheaters (red circles), and weakly competitive cheaters (blue circles). Metacommunity dynamics depicted are: (i) birth or colonisation of empty habitat by host individuals at rate c , (ii) death or loss of existing host individuals at rate e (with simultaneous extinction of local populations residing within the host), (iii) colonisation of new hosts by microbes at rate β , (iv) local extinction of existing microbial populations within hosts at rate δ .

microbial species have identical carrying capacities when on their own, and the resource use of each species is defined by a utilisation curve that falls along a single linear resource gradient (Gaussian resource utilisation curves with standard deviation $\sigma = 1$). The resource niches of x and z are identical to each other – that is, they perfectly overlap – while the resource niche of the weak cheater y is offset from that of x and z by niche distance d , defined as the distance between the means of the niche curves measured in standard deviations (Fig. 1a). Hosts colonised by mutualistic symbionts x benefit from a lower mortality rate e_X relative to that of mutualist-free hosts, e , allowing local populations of mutualists (and any microbial species that can coexist in a host with it) to have a higher lifetime coloniser output, and thus a metacommunity-level fitness advantage due to the longevity of their host (Metz & Gyllenberg 2001; Massol *et al.* 2009).

Since the mutualist provides this benefit to the host at a cost, it is assumed to be competitively inferior to both strongly competitive and weakly competitive cheaters (henceforth, ‘strong cheater’ and ‘weak cheater’), neither of which affects host fitness. This means that because the mutualist and strongly competitive cheater are both identical in their resource use, we assume the competitively dominant strong cheater z will immediately displace mutualists in any local host (hence its description as ‘strong’) as it achieves carrying capacity.

The weakly competing cheater y , like the strongly competing cheater z , is also competitively dominant to the mutualist, and as a result its local equilibrium density is not affected by the presence of the mutualist. However, because the mutualist and weak cheater only partially overlap in resource use (depending on the niche distance between them), the weak cheater y will only reduce the local abundance of mutualist x according to the amount of resource left after y has established itself at carrying capacity (Fig. 1a). Thus, unlike the strongly competing cheater, the weakly competing cheater y can potentially coexist with mutualist x in a host. Strong and weak cheaters, on the other hand, are competitively equivalent, and thus the outcome of competitive interactions between them will be defined by a simple Lotka–Volterra resource competition model (MacArthur & Levins 1967; see Appendix S1), which results in both species being similarly reduced in abundance when present together to the degree that their niches overlap. For the results shown, we consider two versions of the weak cheaters, y_1 and y_2 , whose niches are equidistant from and symmetrically arranged around the mutualist/strong cheater niche (Fig. 1a).

The assumption that the mutualist is immediately excluded within hosts by the strongly competitive cheater is reasonable given the large differences in the scale of the temporal dynamics of microbes (e.g., hours–days) vs. that of hosts (e.g., months–years). Allowing for the transient coexistence of the mutualist with the strong cheater within hosts (due to slow exclusion of mutualists) would likely only add to the overall metacommunity-level fitness of the mutualist and thus promote its persistence (e.g., Pillai *et al.* 2012). Thus, our strict assumption of rapid competitive exclusion of the mutualist represents a ‘worst case’ scenario that ensures our results apply whether competitive exclusion occurs immediately or gradually.

The outcome of competitive interactions within a single host determines what type of communities will be observed at the regional (metacommunity) scale. For example, with four microbial species (x, z, y_1, y_2), and noting that x and z cannot coexist together, there are eleven possible microbial communities, excluding the empty host community with no microbes; i.e., $\{x\}$, $\{z\}$, $\{y_1\}$, $\{y_2\}$, $\{y_1, x\}$, $\{x, y_2\}$, $\{y_1, z\}$, $\{z, y_2\}$, $\{y_1, x, y_2\}$, $\{y_1, z, y_2\}$, and $\{y_1, y_2\}$. Note that in all figures mutualist host occupancy or density refers to the total density of hosts containing mutualist populations, i.e., $\{x\}$, $\{x, y_2\}$, $\{y_1, x\}$, and $\{y_1, x, y_2\}$. The same holds when referring to total weak cheater and strong cheater host-occupancy.

All microbial species have identical baseline rates for colonisation of new hosts, β , and for local extinction rates within existing hosts, δ (Fig. 1b). However, the local equilibrium density that each microbe achieves in a given type of community will have the effect of scaling these rates. Specifically, the lower density experienced by mutualist x in the presence of a weak cheater y reduces the number of x colonisers dispersed from an $\{x, y\}$ community, while increasing the likelihood that the local subpopulation of the mutualist will undergo stochastic extinction δ_x . This means that although the weak cheater will not immediately exclude the mutualist from a local host, it can significantly reduce its local viability and its ability to colonise new hosts, depending on the niche distance or overlap between x and y . Similarly, the positive fitness effect the mutualist will have on host mortality e_X will be reduced to the degree that the local density of x is reduced by y , with e_X approaching e as the density of x approaches 0.

In the case where a mutualist occurs in a mixed community (i.e., with y_1 and/or y_2), the reduction in the mutualist’s local density via competition will lead to its metacommunity-scale colonisation (β) and extinction (δ) rates, as well as the host loss rate e_X , to scale as follows:

$$\begin{aligned}\beta_{x_{\text{mixed}}} &= \beta \times \left(\frac{x_{\text{mixed}}}{\hat{x}}\right), \\ \delta_{x_{\text{mixed}}} &= \delta_x \left(\frac{x_{\text{mixed}}}{\hat{x}}\right)^{-v}, \\ e_{x_{\text{mixed}}} &= e_X + (e - e_X) \left[1 - \left(\frac{x_{\text{mixed}}}{\hat{x}}\right)^u\right],\end{aligned}$$

for scaling exponents u and v , where x_{mixed} and \hat{x} represents the local densities of x in a mixed community (i.e., $\{y_1, x\}$, $\{x, y_2\}$ or $\{y_1, x, y_2\}$), and at carrying capacity, respectively. Thus, the parameters $\beta_{x_{\text{mixed}}}$, $\delta_{x_{\text{mixed}}}$, and $e_{x_{\text{mixed}}}$ correspond to the parameters β_x , δ_x and e_x , but for cases mutualists occur in mixed communities. An equivalent scaling of β and δ parameters occurs for strong and weak cheaters when they are found in mixed communities. For all results shown, $v = 1$.

A simple version of the metacommunity model with only mutualists and strongly competitive cheaters (i.e., a low microbial diversity metacommunity without y_1 or y_2) allows us to explore the likelihood that mutualist symbionts will persist in the face of direct competition from a strong cheater. The simple metacommunity model is given by the following ordinary differential equations:

$$\frac{dH}{dt} = cH(1 - H) - eH - (e_X - e)X,$$

$$\frac{dX}{dt} = \beta X(H - X - Z) - (e_X + \delta)X - \beta ZX,$$

$$\frac{dZ}{dt} = \beta Z(H - Z) - (e + \delta)Z.$$

The upper case letters H , X , and Z represent the total host densities, as well as the densities of mutualist-occupied hosts, and strong cheater-occupied hosts, respectively. This simple metacommunity model was studied using a closed form equilibrium solution for the equation system defined above.

When weakly competitive cheaters are incorporated into the model, the system of equations describing the metacommunity becomes much more complicated. In order to study a holobiont metacommunity with weak cheaters, we implemented our model as a system of ordinary differential equations, with each equation in the metacommunity model representing the change in density of hosts with a particular type of community: six

equations in the one weak cheater model, and nine equations in the two weak cheater model (see Appendix S2 for details). The results of the more the complex metacommunity were solved numerically in MATLAB using functions ode45 and ode15s for periods ranging from 10 000 to 50 000 time steps, which were sufficient to attain equilibrium dynamics.

RESULTS

When the baseline microbial colonisation rate β is relatively high in a simple metacommunity with a strongly competitive cheater, the mutualist will be unable to persist, even when the mutualist gains a metacommunity-scale advantage by reducing the host's loss rate e_X to extremely low values (Fig. 2a). Here, the cheater prevents the mutualist from persisting by rapidly displacing it from hosts. When microbial colonisation rates β are low enough to reduce the rate at which the cheater

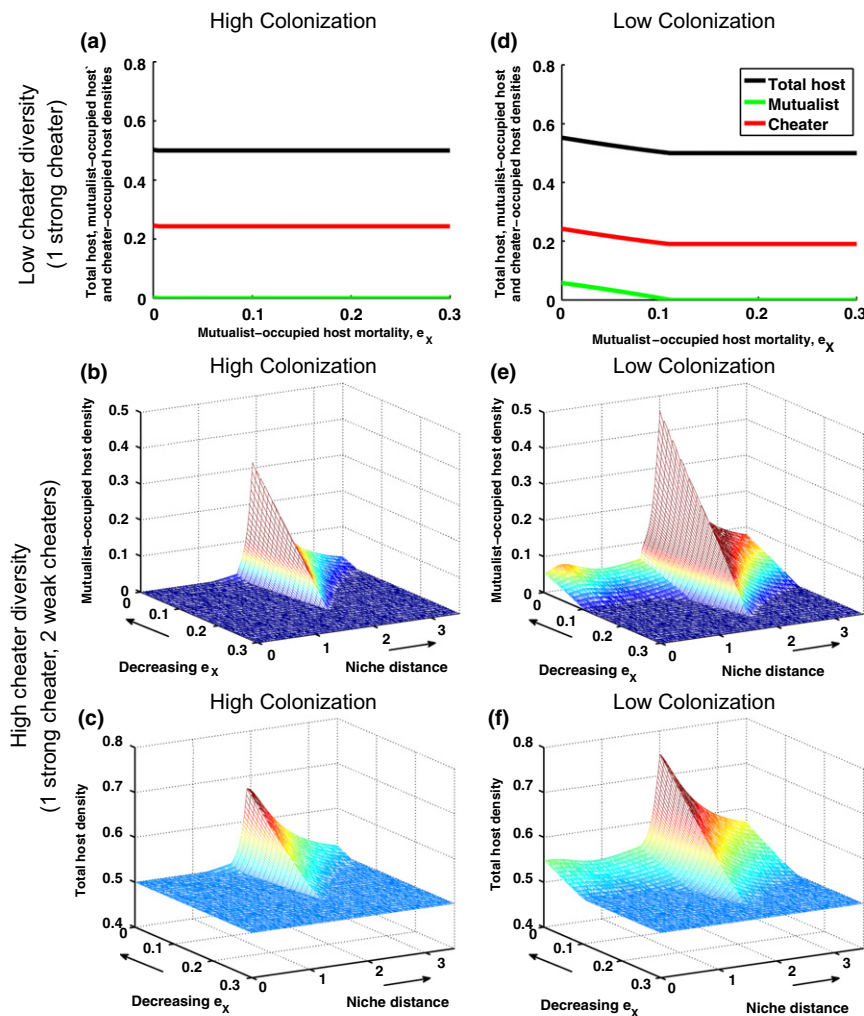


Figure 2 Effects of cheater diversity on metacommunity occupancy of mutualists and host. *Note:* e_X is the mortality of mutualist-occupied hosts, and thus decreasing e_X represents increasing positive effects of mutualist on host fitness. $\beta = 1.21$ for left column panels (a–c); $\beta = 1.0$ for right column panels (d–f). (a, d) Metacommunity with low cheater diversity (one strong cheater). Curves show equilibrium values for total host (black), mutualist-occupied hosts (green), and strong cheater-occupied hosts (red), over range of e_X values. (b, e) Metacommunity with high cheater diversity (two weak cheaters). (b, e) Mutualist-occupied host densities, and (c, f) total host densities shown as e_X and niche distance d between the mutualist and weak cheater varied. All simulations: $c = 0.6$, $e = 0.3$, $\delta = 0.01$, $v = 1$ and $u = 5$.

encounters and excludes the mutualist in hosts, the mutualist can potentially persist in the metacommunity so long as the mutualist has a sufficiently strong positive effect on the density of the host by increasing the host's lifespan (reducing e_X ; Fig. 2d).

This ability of the mutualist to persist in the host population alongside the cheater over a limited parameter range, despite exclusion within individual hosts arises due to an emergent competition-longevity trade-off between the mutualist and the strongly competitive cheater. Under this competition-longevity trade-off, the mutualist essentially engages in niche construction for the cheater by increasing (i) the overall density of the host, (ii) the total habitat available for the cheater, and ultimately (iii) the cheater's host-occupancy (red line, Fig. 2d). These basic patterns demonstrate a well-known result (Palmer *et al.* 2003): that simple colonisation-extinction dynamics within a patch-dynamic framework can *potentially* allow for the persistence of a mutualist and its coexistence with a cheater, but only when it invades as an already fully formed, strong mutualist with a sufficiently high positive effect on the host's fitness (Fig. 2d). However, because the mutualist's beneficial effect on the host's fitness must already be sufficiently strong at the outset, a competition-longevity trade-off is unlikely to explain the initial emergence and stabilisation of weak beneficial traits that only later evolve into stronger beneficial traits within the holobiont.

When the weak cheaters y_1 and y_2 are present in the microbial metacommunity, the mutualist not only persists, but its host-occupancies (middle row, Fig. 2b,e) are now significantly higher than expected when only the mutualist and strongly competitive cheater are present. The host occupancy of the mutualist also shows a peak at intermediate niche distances or niche overlap (intermediate d) between the mutualist and the weak cheaters (Fig. 2b,e). This peak in mutualist host density at intermediate niche distances leads to a concomitant peak in the total density of hosts (Fig. 2c,f) because of the positive effect of the mutualist on host lifespan. At intermediate niche distances, the weak cheater allows the mutualist to persist by reducing the direct negative effects of the strong cheater on the mutualist by competitively suppressing the strong cheater's host-occupancy at the metacommunity (regional) scale, thus decreasing the rate at which the mutualist encounters the strong cheater in local hosts. Here, the *indirect* positive effects of the weak cheater on the mutualist (through its competitive suppression of the strong cheater's host occupancy) trump its *direct* negative competitive effects on the mutualist. The indirect positive effects of the weak cheater are so strong that they allow the mutualism to persist even when the mutualist's beneficial effects on the host are weak (i.e., e_X not very different than e ; see also Fig. S1). However, large niche distances (low overlap) reduce the weak cheater's ability to competitively suppress the metacommunity-scale host-occupancy of the strong cheater, thus eroding the former's indirect positive effects on the mutualist. Conversely, small niche distances (high overlap) increase the direct negative effects of the weak cheater on the mutualist despite the indirect positive effects arising from the suppression of the host-occupancy of the strong cheater. Under this scenario, the costs of the direct negative effects of the weak cheater trump the benefits of its

indirect positive effects, thus causing the mutualist to either become rare or go extinct (Fig. 2b,e).

Note that the balance between direct negative and indirect positive effects required for the persistence of the mutualist can only arise when there is sufficient spatial structure or heterogeneity in the host-microbiome metacommunity. When microbial colonisation rates β are sufficiently high (e.g., $\beta = 2.5$ with the parameters used in Fig. 2), the implicit spatial structure of the host-microbiome metacommunity collapses and the dynamics becomes equivalent to those of a well-mixed system. Under such conditions, the strong and weak cheaters rapidly encounter and exclude the mutualist from local hosts, leading to its extinction across the entire metacommunity.

In effect, niche distance controls the relative importance of the weak cheater's direct negative effects and indirect positive effects on the mutualist (Fig. 3). When niche distance is low or near zero (i.e., complete niche overlap between mutualist and weak cheater), the weak cheater has a large direct negative effect that overwhelms its strong indirect positive effect on the mutualist, such that the weak cheater essentially behaves like a strong cheater by rapidly excluding mutualists from local hosts and reducing mutualist host-occupancy in the metacommunity (Fig. 3a). When niche distance is very high, although the weak cheater now has a small direct negative effect on the mutualist, it also has a small direct negative effect on the strong cheater, and thus only a small indirect positive effect on the mutualist. As a result, the strong cheater can return to the metacommunity host-occupancy it enjoys in the absence of the weak cheater, thus allowing it to competitively displace the mutualist (Fig. 3c).

Overall, mutualist persistence and host-occupancy depends on niche distance and its influence on the balance between the direct and indirect effects of the weak cheater: niche distance has to be large enough so that the weak cheater is not capable of rapidly excluding the mutualist from hosts (i.e., weak direct negative effect on the mutualist), but not so large as to prevent the weak cheater from being able to suppress the strong cheater's regional host-occupancy (i.e., strong indirect positive effect on the mutualist; Fig. 3b). A consequence of this balance between positive and negative effects at intermediate niche distances is that the evolution of beneficial traits in the mutualist will likely lead selection to ultimately maximise mutualist and host density at intermediate niche distances (see Appendix S3 for the adaptive dynamics of beneficial traits in a system with one weak cheater). Because weak cheaters in our model help to suppress strong cheaters by filling up the available resource niche within hosts, niche distance provides a potential proxy for estimating the effects of more diverse microbiomes. Thus, cases of high species packing in rich or saturated communities should correspond to intermediate niche distance, whereas cases of relatively low diversity and sparsely filled niches would likely correspond to high niche distance.

In the results presented so far, the strong cheater represents a commensal microbe that has no effect on the host's fitness. However, when the strong cheater is modeled as an actual

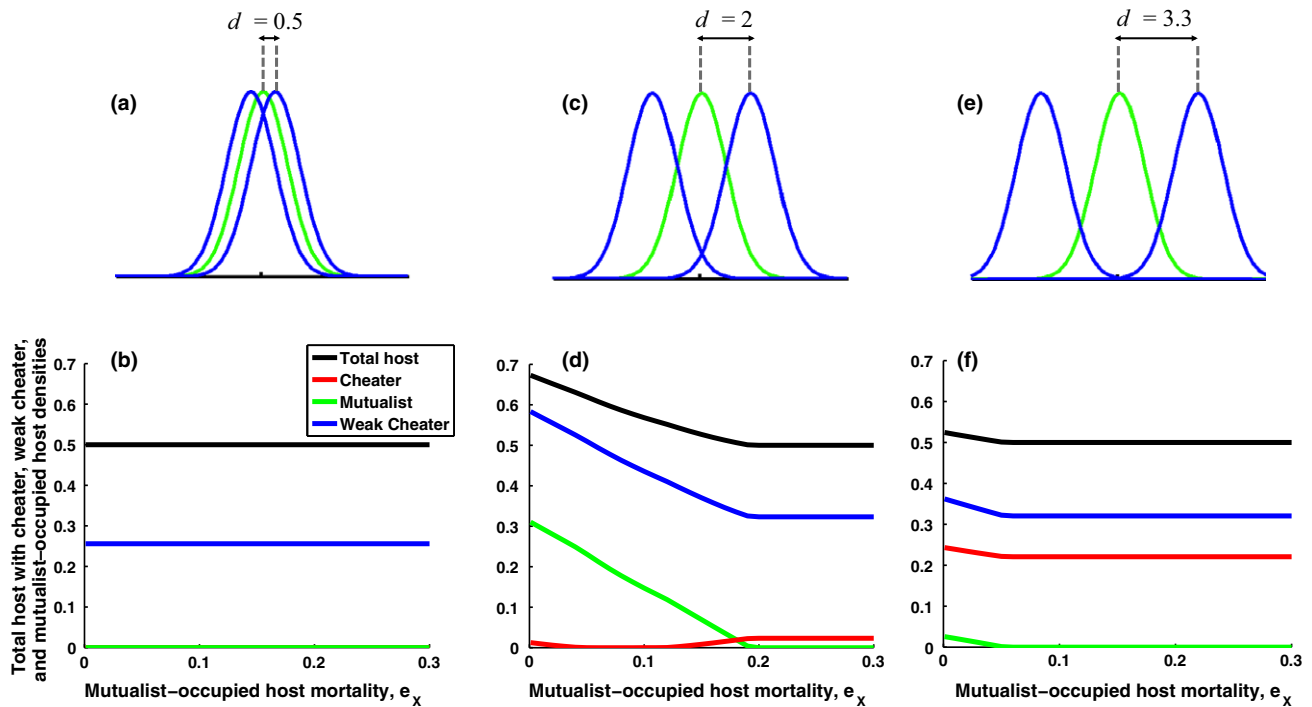


Figure 3 Effect of niche distance d on the ability of the weak cheater to facilitate mutualist. (a, c, e) Different niche distance d values between the weak cheater and the mutualist/strong cheater, and the corresponding (b, d, f) equilibrium densities for total host (black), mutualist-hosts (green), strong cheater-hosts (red), and weak cheater-hosts (blue) over a range of e_x values. Weak cheater-occupancies are calculated by summing total hosts occupied by y_1 or y_2 , or both. (a, b) At low d , the weak cheater behaves like a strong cheater, suppressing the mutualist for even low e_x . (c, d) At intermediate d , the weak cheater facilitates the persistence of the mutualist over a significant range of e_x . (e, f) At high d , the weak cheater is unable to effectively suppress the strong cheater through competition, so the mutualist declines as the strong cheater recovers. Results from simulations shown in Fig. 2b,c.

pathogen that actively reduces the host's fitness, weak cheaters are also able to suppress the regional (metacommunity) density of the pathogen via resource competition, leading to the same qualitative patterns of mutualist and host densities observed when the strong cheater represents a commensal microbe (see Appendix S4).

DISCUSSION

We have shown that the combination of resource competition within the microbiome and metacommunity-scale dynamics gives rise to an emergent indirect positive interaction between the mutualist and the weak cheaters that is mutually beneficial. Indeed, by coexisting with the mutualist within hosts, weak cheaters gain a metacommunity-scale fitness advantage over strong cheaters by benefiting from the mutualist's positive effect on host longevity (Fig. S1b). At the same time, the mutualist indirectly benefits from the weak cheater's ability to competitively suppress the strong cheater, despite suffering the direct costs of competition with the weak cheater.

These results demonstrate that resource competition within diverse microbial communities can promote rather than prohibit the emergence, evolution and stabilisation of mutualisms within the microbiome. The simple and parsimonious (meta) community-based mechanism that underlies this positive effect of competition on the persistence of mutualisms is unique in that it does not rely on the ability of the host to selectively distinguish between potential mutualists and cheaters for tar-

geted rewards and sanctions (Denison 2000; Yu 2001; Palmer *et al.* 2003; Stanton 2003). Instead, this mechanism merely relies on the joint effects of two critical features of most natural holobiont systems: (local) within-host competitive interactions within the microbiome combined with metacommunity-scale dynamics of the holobiont. Both of these features are required in order for beneficial traits to emerge from relatively weak or neutral microbial–host interactions.

Current theory suggests that spatial processes in the form of metacommunity trade-offs can reduce the deleterious effects of microbial competition on mutualism and thus allow the coexistence of mutualists and cheaters (Yu 2001; Hoeksema & Kummel 2003; Palmer *et al.* 2003). However, we have shown that such trade-offs only allow the persistence of mutualism when the beneficial effects of the mutualist are established and strong at the onset (Fig. 2d). In such cases, the cheater will still likely remain at high host-occupancies relative to the mutualist, and may dominate (Fig. 2d). Hence, while space alone can allow the persistence of mutualism under certain limited conditions (pre-existing and strong mutualistic relationship), it cannot explain the relatively high abundance of mutualists and the rarity of cheaters or pathogens in natural microbial communities (Huttenhower *et al.* 2012). Our results turn the standard explanation for the persistence of mutualism on its head: space is not a general solution to the disruptive effect of competition, while resource competition can have a net constructive, rather than disruptive effect on the persistence of mutualisms within microbial guilds. This constructive

effect of competition demonstrates not only how relatively weak mutualists can arise, but also how strong cheaters such as parasites and pathogens can be kept at low host-occupancy levels so as to not predominate in the holobiont, thus generating patterns of microbial abundance that are consistent with those observed in most natural systems (e.g., Stat *et al.* 2008). Overall, this suggests that diversity within microbial communities – and not just the diversity of host communities (Schmidt & Ostfeld 2001; Johnson *et al.* 2013) – can play a major role in reducing disease outbreaks.

What is important to note about these theoretical results is how the patterns of local within-host microbial abundances differ drastically from what is expected with the standard mechanisms involving cheater inhibition through host selection or interference competition (Fig. 4a,c). Both our model and the standard mechanisms predict that the regional (metacommunity) scale densities of hosts occupied by beneficial microbes should be relatively high, while hosts occupied by strongly disruptive cheaters should be low (Fig. 4b,d). For the standard models, this regional (metacommunity) scale pattern arises because of the suppression of strongly competitive cheaters directly *within* individual hosts via targeted inhibition (Fig. 4a). Our results are based on the more general assumption of indiscriminate microbial resource competition, which – when all the relevant species are present – will lead to the opposite scenario at local (within-host) scales, whereby strong cheaters are abundant and mutualists are rare or excluded (Fig. 4c).

In other words, in the case of host selection or interference competition, the predominance of the mutualist at the regio-

nal (metacommunity) scale is simply a direct extension of the relative abundances observed at the local (within-host) scale. In contrast, the novel metacommunity-level mechanism that we have described would give rise to (i) the suppression of the mutualist at local (within-host) scales in the presence of all microbial cheaters, but (ii) the paradoxical persistence of the mutualist and the suppression of the strong cheater at the regional (metacommunity) scale (Fig. 4c,d). These divergent patterns of microbial abundance at local vs. regional scales arise in part due to resource competition between microbes within diverse guilds, thus demonstrating how biodiversity – through its promotion of resource competition – can play a critical role in the maintenance of mutualisms. However, this constructive effect of biodiversity and resource competition is likely to be cryptic in natural systems, only becoming evident when the dynamics of holobionts are considered simultaneously at both local and regional scales.

This stark difference in the local and regional-scale patterns predicted by the two mechanisms may offer us an opportunity to experimentally discern or partition the relative importance of both targeted inhibition (host selection and interference competition) within hosts, and the more general resource competition and metacommunity-based mechanism that we have identified. This would require a series of manipulative experiments to (i) ascertain the relevant functional groups (e.g., mutualists vs. cheaters) within the microbial community and (ii) study their community-level interactions (Fig. 5). This could be achieved by comparing the effects of various monocultures and mixtures of microbial species on host fitness. Host

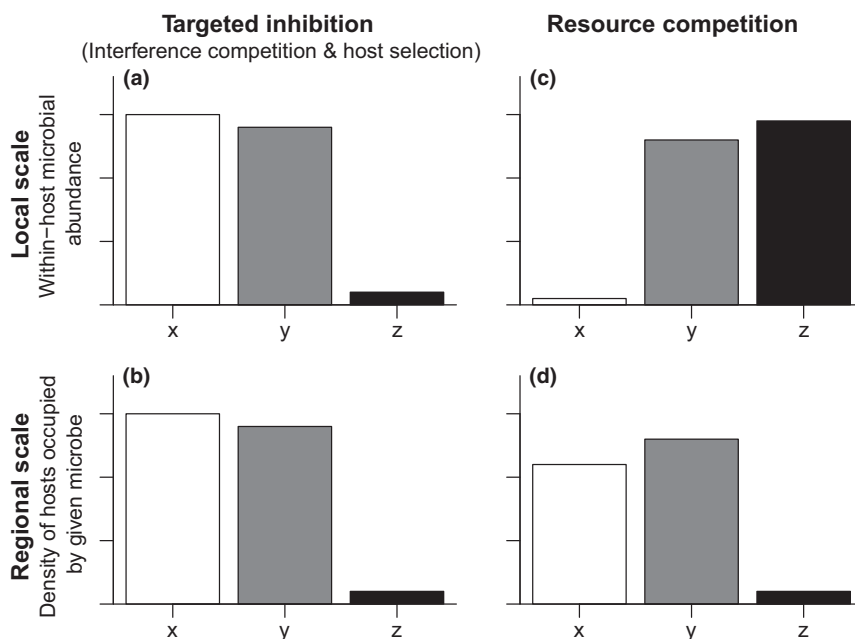


Figure 4 Microbial abundance predictions of the standard model involving inhibition of cheaters through interference competition and host selection (left column), and our metacommunity model utilising resource competition (right column). The top row shows predictions for abundances of mutualist (x), weakly competitive cheater (y), and strongly competitive cheater (z) microbes within hosts. The bottom row shows predictions for densities of hosts occupied by each type of microbe. In the standard model (a, b), strongly competitive cheaters are suppressed directly within hosts, while in our model with resource competition (c, d), strongly competitive cheaters are kept at low densities at the regional scale through metacommunity dynamics. This generates very different predictions for the within-host abundances (a, c), despite both models predicting a qualitatively similar pattern of strong cheater suppression at the regional scale (b, d).

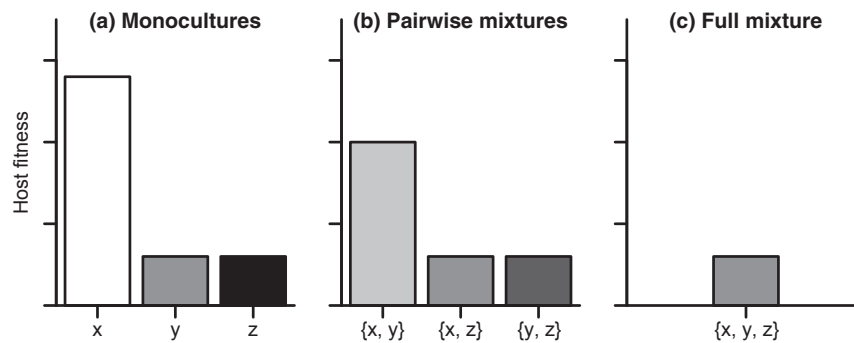


Figure 5 Predictions for how the different microbial functional groups described in the model (mutualist x , weakly competitive cheater y , and strongly competitive cheater z) will affect host fitness. The fitness response of hosts inoculated with different microbial community mixtures can be used to sort candidate microbial taxa in natural systems. (a) Here, strong and weak cheaters represent commensal microbes (no affect on host fitness), such that the fitness of an empty host is equivalent to one containing y or z monocultures. (b) Weak cheaters are functionally defined by their effect of partially reducing the positive fitness effect of mutualists when in mixtures without strong cheaters (b), while strong cheaters are functionally defined by their effect of completely eliminating the positive fitness effect of mutualists whenever mixed with mutualists (b, c). See Fig. S2 for the case where the strong cheater is a pathogen.

fitness would be low in monocultures of weak or strong cheaters, but high in monocultures of mutualists (Fig. 5a). Mixtures consisting of various combinations of mutualists, weak cheaters and strong cheaters could be used to distinguish weak cheaters from strong cheaters, with weak cheaters partially reducing and strong cheaters completely negating the positive effects of mutualists on host fitness (Fig. 5b,c).

The constructive role for resource competition identified here has important implications for understanding how holobiont systems may respond to disturbances such as disease outbreaks or environmental change. For example, the finely tuned regulatory systems that are assumed to be maintaining mutualistic interactions within coral microbial communities are predicted to be particularly prone to disruption by environmental stresses such as higher temperatures due to climate change (Rosenberg *et al.* 2007; Bourne *et al.* 2009). Although microbes may be able to evolve rapidly enough to respond to changing environmental conditions, their slow-growing coral hosts are unlikely to do so (Baskett *et al.* 2009, 2010). This has led to predictions of possible mass die-offs of corals within decades due to both higher temperatures and the rise of microbial pathogens (Altizer *et al.* 2013). Our work suggests that new mutualistic relationships can emerge spontaneously through metacommunity-level dynamics, even when corals are unable to regulate their own microbial community by using host selection to exclude cheaters and pathogens or acquire novel beneficial microbes needed to weather stressful environmental conditions. Such a mechanism could potentially allow corals and similar holobiont systems experiencing stressful environmental conditions the time needed to evolve the regulatory system required to promote and stabilise mutualist microbial species within their respective microbiomes.

The constructive effect of microbial competition has immediate consequences for the manner in which holobiont systems are studied empirically. Indeed, the (meta)community-level mechanisms that govern the health and fitness of holobiont systems are unlikely to be detected using classic, autecological approaches that focus on identifying the effects of individual

microbial species on their host. Hence, if community-level interactions within the microbiome are responsible for maintaining mutualisms and blocking cheaters or potential pathogens, then current experimental approaches that focus on the direct effects of a single mutualist on host fitness will be unable to explain the persistence of mutualists or the occurrence of disease outbreaks. For example, the predominance of autecological approaches may partly explain the difficulties in determining the etiology of many diseases afflicting corals and our inability to associate various coral disorders with a single pathogen (Rosenberg *et al.* 2007; Bourne *et al.* 2009). Similarly in the case of humans, community-level microbial interactions within the gut microbiome appear to play a significant role in determining whether healthy or disease states arising from dysbiosis manifest themselves (Mazmanian *et al.* 2008; Round & Mazmanian 2009). Our results suggest that competitive interactions within the microbial community can suppress pathogens at larger spatial scales and, in doing so, lead to the context-dependent emergence of diseases in holobionts. Among-host variation in microbial community structure may thus provide an explanation for the seemingly paradoxical observation that the presence of a pathogen compromises the health of some hosts but not others.

The (meta)community-level mechanism responsible for the maintenance of mutualists and the suppression of cheaters and potential pathogens is strongly dependent upon significant niche overlap between the various cheaters present in the microbiome. Hence, a significant amount of the microbial diversity that plays a critical role in maintaining microbial mutualisms may be obscured by hidden genetic variation among closely related (and strongly overlapping) strains that have not been sufficiently resolved into distinct OTUs. In those cases where niche traits are phylogenetically conserved (Cavender-Bares *et al.* 2009), the detection of the *cryptic role* played by microbial diversity may then ultimately hinge upon our ability to resolve the *cryptic variation* in microbial communities (Koeppl & Wu 2013). Therefore, not only do our results suggest the need for a more synecological approach that is capable of accounting for community-level interactions

within microbiomes at multiple scales, but they also underscore the importance of current attempts at providing a more thorough accounting of the true range of functional variation in microbial communities (e.g., Huttenhower *et al.* 2012).

The emergence and persistence of mutualism via competitive interactions within the holobiont demonstrates a critical, but cryptic role of microbial diversity. Despite diversity being associated with the *direct* negative effects of resource competition from multiple potential cheaters, it can also entail *indirect* positive effects that arise as competition amongst multiple cheaters suppress the more detrimental effects of strong cheaters. Hence, understanding the evolution and persistence of mutualism requires a shift to (meta)community approaches that explicitly incorporate higher order interactions between microbes and their host across spatial scales.

AUTHORSHIP

P.P. formulated the model, performed the simulations, analysed the data/results and wrote the manuscript; T.G. helped analyse the results and co-wrote the manuscript; S.V. helped analyse the results and edit the manuscript.

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