

CHAPTER 6

Specialised mutualistic services or providing it all? The evolution of division of labour among mutualistic symbionts

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Introduction

While a key question in evolutionary biology is how mutualisms evolve (West *et al.*, 2007b; Leigh, 2010), usually, studies ask *why* a potential mutualist cooperates, not *how* it should cooperate. Rather than focusing on the optimal *level* of cooperation, we here study the optimal *nature* of mutualist cooperation. We are interested in factors that govern if mutualists specialise on providing a single mutualistic service, or generalise and provide various different benefits. Thus, we are interested not only in when cooperation between species *per se* is maintained, but in the specialisation or generalisation of cooperation, and in potential interactions between conditions favouring mutualism maintenance and its specialisation or generalisation.

Traditionally, mutualisms have been analysed as pairwise interactions. However, many hosts interact with multiple mutualists (Palmer *et al.*, 2003; Stanton, 2003; Dubilier *et al.*, 2008; Afkhami *et al.*, 2014). These mutualistic communities can provide a wide range of resources and services to hosts. For instance, plants may interact simultaneously with various insect species providing pollination services, with protective ants deterring herbivores and with diverse nutritional root mutualists (Mack & Rudgers, 2008; Ohm & Miller, 2014; Barber & Soper Gordon, 2015; van der Heijden *et al.*, 2015). Multiple mutualists can influence not only host benefits from mutualism (McKeon *et al.*, 2012; Afkhami *et al.*, 2014; Fraune *et al.*, 2015; van der Heijden *et al.*, 2015), but also the ecological dynamics and evolution of the mutualists (Palmer *et al.*, 2003; Whitney & Rudgers, 2009; Lee & Inouye, 2010; Larimer *et al.*, 2014), including the mutualistic traits potential partners evolve (May & Nelson, 2014). For instance, the degree to which different mutualists provide similar or different services, or the overlap in mutualists benefits, can vary (Afkhami *et al.*, 2014). Some mutualists can be thought of as generalists and provide their hosts with various diverse benefits (services or resources). Arbuscular mycorrhizal fungi provide plants with a range of different benefits such as nutrients, pathogen protection and drought resistance (Newsham *et al.*, 1995; Augé, 2001; Sikes *et al.*, 2010; Harman, 2011) and are an example of such generalist mutualistic symbionts (sometimes this is called multifunctionality). Similarly, mutualistic ants protect their plant hosts from herbivores, but some also prevent bacterial pathogens from infecting the plants (González-Teuber *et al.*, 2014). In contrast, many other mutualists are highly specialised and provide only a single benefit. Examples of specialised mutualists include pollinators (Davis *et al.*, 2014), cleaners (Bshary & Grutter, 2005; Nunn *et al.*, 2011), seed dispersal (Martin Schaefer *et al.*, 2007), and symbiotic nitrogen fixing bacteria, for instance in legume plants (Denison, 2000; Foster *et al.*, 2011).

If a host needs multiple specialised mutualists or symbionts to obtain its full range of mutualistic services, we can say there is a division of labour among those mutualists

(Shapiro, 1998). Mutualists may then also be considered indirect mutualistic partners to each other (Stachowicz & Whitlatch, 2005), because they benefit each other by sustaining a shared host. The distinction between specialised and generalist mutualists is important, because it has important consequences for the evolutionary dynamics of the whole interaction. For instance, while a division of labour may result in more efficient provisioning of mutualistic services, specialised mutualist communities could be more sensitive to the (temporary) absence of key mutualistic species (Palmer *et al.*, 2003) or to environmental variation (Condon *et al.*, 2014) or could constrain host diversification (Raimundo *et al.*, 2014). Competition and coexistence of mutualists can be affected by their number and similarity (Palmer *et al.*, 2003; Jones *et al.*, 2009, 2012). Mutualist specialisation may also impede the evolution of mutualist cooperation with the host, because specialised communities may require more mutualist types, potentially driving conflicts among them (Frank, 1997; Poulsen & Currie, 2010; Friesen & Mathias, 2010). Thus, potentially there is a conflict because the conditions favouring a division of labour among mutualists may not be those favouring stable cooperation between hosts and mutualists.

Both theoretical and empirical work has explored when mutualists provide benefits to a single host species (host specialisation or specificity) or multiple host species (*e.g.* Bascompte *et al.*, 2003; Kawakita *et al.*, 2010; Schleuning *et al.*, 2012; Frick *et al.*, 2013; Brosi & Briggs, 2013; Ehinger *et al.*, 2014), or when we expect divisions of labour between hosts and symbionts (Schwartz & Hoeksema, 1998; Grman *et al.*, 2012), yet we know little about why specialisation or generalisation of services evolves *among* mutualists. One idea is that generalist mutualists are more valuable to the hosts because they provide various services, and may even be preferentially selected (Chapter 2; Werner *et al.*, 2014c). Alternatively, specialists may more efficiently provide benefits to the host, allowing them to outcompete generalist mutualists. This is the idea that a jack-of-all-trades is a master of none, because providing various benefits to hosts may require very different adaptations, representing a strong trade-off (Wilson & Yoshimura, 1994; Østman *et al.*, 2014; Bruns *et al.*, 2014). Strong trade-offs between different benefits could favour specialisation and division of labour.

Why are some mutualists and symbionts favoured to be generalists providing a range of different services or resources? When is a division of labour among specialised symbionts favoured? And can active host strategies, like partner choice or rewarding of cooperative behaviour affect the potential for division of labour? We develop a range of evolutionary game theory models studying these questions. In our models, we study the evolution of division of labour and cooperation level in potentially mutualistic symbiont populations and determine the conditions for specialist and generalist symbionts to evolve, and for cooperation between symbiont and host to be maintained. We analyse if the evolution

of a division of labour among symbionts and symbiont cooperation is affected by: (i) the strength of the trade-offs between symbiotic benefits; (ii) symbiont numbers per host; (iii) host rewarding of more cooperative symbionts; and (iv) partner choice by the host.

Modelling and Results

Host and symbiont life cycle

We consider a host that needs to obtain two resources (resource A and resource B) from a population of mutualistic symbionts. We will refer to these as ‘symbionts’ throughout our manuscript, but in principle our model also extends to free-living mutualists. Also, while we use the term ‘resources’, our model similarly applies to mutualistically provided services or other benefits. For instance, our model could refer to a host plant that needs to obtain two different soil nutrients, or a soil nutrient and water, but also to an insect host that obtains both nutritional and defensive benefits from its symbiont community (Oliver *et al.*, 2008; Łukasik *et al.*, 2013). In our model, hosts can only obtain resources A and B through symbiosis, not directly from the environment. Symbionts can transfer both resource A and B to the host (generalist symbiont) or specialise in providing either A or B (specialist symbionts). This is controlled by the parameter α , with $\alpha = \frac{1}{2}$ representing a generalist transferring equal amounts of both resources and $\alpha = 1$ or $\alpha = 0$ respectively representing symbionts fully specialised on transmitting resource A or B (Table 1). Symbionts also have an outside option, meaning they have the potential to evolve a non-mutualistic lifestyle without investment in and interaction with hosts. This allows us to determine the conditions in which entering into mutualism is favourable to symbionts, *i.e.* the conditions under which cooperation is stabilised or breaks down, and to establish if cooperation stability is affected by divisions of labour among symbionts.

Table 1: Definition of parameters

A	Environmental availability of resource/service A ($0 < A \leq 1$).
B	Environmental availability of resource/service B ($0 < B \leq 1$).
n	Number of symbionts per host additional to the focal individual ($n \geq 1$). The total number of symbionts per host is thus equal to $n + 1$.
α	Symbiont specialisation in providing resource A vs. resource B ($0 \leq \alpha \leq 1$).
β	Strength of trade-off between transmitting resource A and B (complementary transmission: $0 < \beta < 1$, antagonistic transmission: $\beta > 1$).
x	Symbiont level of cooperation ($0 \leq x \leq 1$).
s	Strength of host selection of symbionts ($s > 0$).
$F_{Outside}$	Fitness of the symbionts ‘outside’ option, <i>i.e.</i> the fitness a symbiont would have if it would live asymbiotically, without interacting with a host. Since we only model the evolution of symbiont traits when in symbiosis, this is a fixed parameter.

We assume that in each generation the host selects $n+1$ symbionts from the environment to provide it with its symbiotic needs. Each host selects at least two symbionts (*i.e.* $n \geq 1$), ensuring there is a potential for division of labour to evolve. Initially, we will assume that hosts select symbionts from the environment randomly (*i.e.* that they have no information about the resource(s) provided by the symbionts, or cannot act on that information). Later, we will relax this assumption and allow for active host control over the symbiont community. We are interested in the evolution of symbiont trait α , and particularly in the conditions under which we expect a split in two symbiont populations with different values for α to be favoured, *i.e.* a situation where there is division of labour among symbionts. We derive the full equations for symbiont dynamics, the symbiont fitness equations and the symbiont cooperation level in the Appendix (Equations A1-A4).

Briefly, we assume a population of symbionts consisting of two potential types, resident and mutant symbionts, interacting with a host. Symbiont numbers change according to a logistic growth model including a term where the focal symbiont's fitness depends on its own strategy and on potential other type's strategy (equations A1). Specifically, symbiont fitness depends on two evolved variables: (i) their level of specialisation and (ii) their level of cooperation x (equation A3), as well as on the phenotypes for these variables of the other symbionts interacting with the same host. Symbiont fitness is maximised if the host's fitness is high, which is the case if hosts receive an adequate supply of both crucial resources. Importantly, the symbionts face a trade-off in transmitting the two resources, such that a symbiont with specialisation α transmits $A\alpha^\beta$ of resource A and $B(1-\alpha)^\beta$ of resource B (equations A2). The parameter β determines the strength of the trade-off between transmitting resource A and B (Figure 1), where $0 < \beta < 1$ means that their transmission is complementary (weak trade-off), while $\beta > 1$ means that their transmission is antagonistic (strong trade-off) (Egas *et al.*, 2004; Parvinen & Egas, 2004; Débarre & Gandon, 2010). When transmission is complementary, transferring an extra unit of resource A to the host reduces the amount of resource B a symbiont can transfer by less than one. In contrast, if there is antagonistic resource transmission investing in transmitting resource A strongly reduces a symbiont's capacity to provide B.

Furthermore, we assume that when in symbiosis, the symbionts face a linear trade-off between cooperating with the host and investing in own reproduction. The symbionts' cooperation level is x , leaving $1-x$ for investment in own reproduction. Thus a symbiont with $x = 0$ does not invest any resources in the host and is thus not a mutualist, while a fully cooperative symbiont ($x = 1$) has a fitness of zero because no resources remain for reproduction (equation A4). We determine optimal strategies assuming that symbionts are favoured to enter into mutualism, and we will then see when cooperative strategies are favoured relative to the outside option.

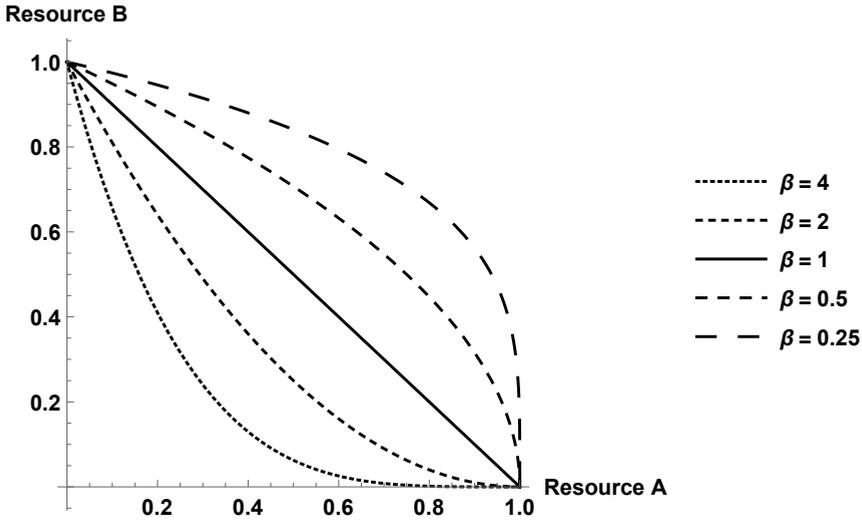


Figure 1: Parameter β determines the trade-off strength between transmitting resource A and resource B. Large values of β give antagonistic resource transmission, while values below 1 give complementary transmission of resources from symbiont to host. The x-axis indicates the relative amount of resource A transmitted, ranging from 0 to 1 indicating only, the y-axis indicates the same for resource B.

Equilibrium resident symbionts are perfect generalists

To determine the evolutionary stable symbiont strategy, we perform an evolutionary invasion analysis (Appendix equations A5-A7; Day, 2000; Otto & Day, 2007a,b). We first study the equilibrium (convergent stable) value of α that is expected to evolve if there is only a single type of symbiont. We find that regardless of the number of symbionts per host (n) or of the trade-off (β), resident symbiont population will always evolve towards a perfect generalist strategy (Appendix equation A6), or towards:

$$\alpha = \frac{1}{2} \tag{1}$$

Thus, if there is only a single symbiont type it will be a perfect generalist transmitting equal amounts of both resources. This is as expected, because we had assumed that the host needs both resource A and resource B to survive, and cannot obtain either directly from the environment. Therefore, symbiont fitness is maximised by providing both resources to the host, because higher host fitness benefits the symbiont population.

Symbiotic division of labour evolves with high symbiont numbers and strong trade-offs

We then perform an evolutionary invasion analysis, to find when symbiont generalism is an evolutionary stable strategy (ESS) and when it is not. We discover that the generalist resident strategy of $\alpha = 1/2$ is invadable by more specialised symbionts whenever (Appendix equation A7):

$$\beta > 1 + \frac{1}{n} \quad (2)$$

If this condition is met, a population composed half of individuals with $\alpha = 0$ and half of individuals with $\alpha = 1$ is uninvadable (Appendix equation A7) and a division of labour among specialised symbionts evolves. Thus, division of labour evolves more easily when transmission of both resources by a single individual is more antagonistic (larger β) because the relative inefficiency of providing both resources simultaneously increases with stronger trade-offs. Also, specialisation is more easily favoured when the number of mutualistic partners (n) increases because the risk of the symbiotic community failing to provide the host with one of the resources diminishes when a host associates with more symbionts.

Mutualism is easier to maintain if symbionts are generalists

We then set out to determine if and how the conditions that favour symbiont specialisation could interact with the maintenance of mutualism. To do so, we established when potential symbionts would be favoured to evolve a mutualistic lifestyle, versus an asymbiotic outside option, for instance a free-living state. If a symbiont can achieve a higher fitness through the outside option, this will be favoured and the symbiont-host mutualism collapses. To determine the conditions under which mutualism can be maintained, we first determined the equilibrium level of cooperation, x^* , when in symbiosis. Since we are interested in the effect of symbiotic division of labour on maintenance of cooperation, we studied this both in the case of a single generalist population, and for two specialised symbiont types (*i.e.* when inequality (2) is met). We found that in both case equilibrium cooperation is given by (Appendix equation A10):

$$x^* = \frac{2}{3+n} \quad (3)$$

Thus, we find that, when in symbiosis, average level of cooperation decreases with increasing number of symbionts (Figure S1). The reason for this is that host benefits for symbionts need to be shared among more other symbionts with increasing n , selecting

for lower symbiont investment in cooperation. Given this conclusion, what are the conditions under which a non-symbiotic lifestyle will be favoured, and symbionts are expected not to interact with the host at all? We find that this differs between generalist and symbiont communities. If symbiont generalism is favoured (*i.e.* inequality (2) is not met), cooperation is maintained if (Appendix equation A11):

$$\frac{AB(1+n)^2}{(3+n)^3 4^{(\beta-1)}} > F_{Outside} \quad (4)$$

While if there are two specialised symbionts types, cooperation is only maintained if (Appendix Equation A13):

$$\frac{ABn(1+n)}{(3+n)^3} > F_{Outside} \quad (5)$$

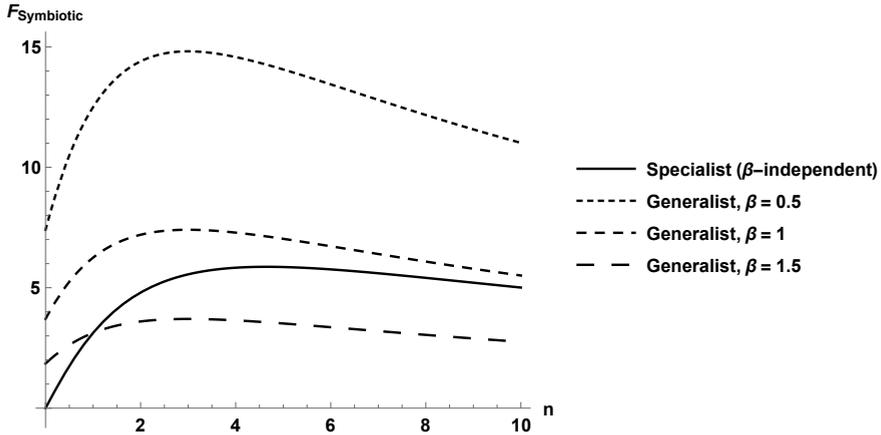


Figure 2: Symbiont cooperation conditions, *i.e.* results (4) and (5).

Symbionts cooperate if $F_{Outside}$, or the fitness associated with their non-symbiotic outside option, is lower than the fitness associated with the symbiotic option $F_{Symbiotic}$, *i.e.* lower than the curves printed here. Thus, a higher curve represents a larger domain over which mutualism is favourable, *i.e.* cooperation being easier to maintain. If there is a division of labour and specialist symbionts (continuous line), $F_{Symbiotic}$ is independent of β because the two specialised symbiont types that evolve will each provide only a single resource. If symbionts are generalists, mutualism is more easily maintained with weaker trade-offs (lower β). If resource transfer is antagonistic ($\beta > 1$), generalists are unlikely to evolve (inequality 2), but if they do mutualism is unlikely to be maintained because of low host fitness due to limited resource transfer. For all curves, $A = 10$ and $B = 10$ in this figure.

From this it follows that mutualism is most easily favoured when the number of symbiotic partners is at an intermediate level (Figure 2). These intermediate optima arise because at high symbiont numbers a tragedy of the commons arises where each individual is favoured to exploit the host whilst relying on others to cooperate (Rankin *et al.*, 2007). Meanwhile, at low symbiont numbers per host, there are not enough resources to make investment in the host worthwhile, favouring the non-mutualistic outside option. This problem is exacerbated when symbionts are specialised, because if there is a division of labour it becomes more likely that the host will not receive enough of one of the two required resources when there are only a few symbionts. Therefore, investing in the host has a significant probability of yielding no benefits in those conditions. In general, this means that mutualism is more easily favoured when symbionts are generalists. However, if resource transmission is antagonistic, $\beta > 1$, two specialists provide more total resources than two generalists. Therefore, if there is antagonistic resource transmission, cooperation may be more easily maintained with symbiont specialisation (Figure 2).

Preferential rewarding enables the high symbiont numbers required for division of labour

We have so far considered cases where host benefits are equally shared among symbionts, *i.e.* where host do not discriminate between more or less cooperative symbionts. However, mutualism can be stabilised if hosts are able to reward more cooperative symbionts (West *et al.*, 2002b; Kiers *et al.*, 2011). Here, we consider a relative rewarding scheme called ‘linear proportional discrimination’, where symbionts are ‘rewarded’ by the host with benefits proportional to their relative contribution to the host’s fitness (Wyatt *et al.*, 2014). We discover that with host rewarding, whether symbionts are generalists or specialists, their optimal level of cooperation when in mutualism is (Appendix Equation A14, Figure S1):

$$x^* = \frac{2 + n}{3 + 2n} \quad (6)$$

This means that optimal level of cooperation stabilises at $x^* = \frac{1}{2}$ at high symbiont numbers n (Figure S1), in contrast to when host benefits are shared among symbionts equally where it drop to zero (result 3). When symbionts are generalists, we find this means that they enter into mutualism when (Appendix Equation A15):

$$\frac{AB(n+1)^2(n+2)^2}{4\beta(2n+3)^3} > F_{Outside} \quad (7)$$

whereas when they are specialists, they enter into mutualism when (Appendix Equation A16):

$$\frac{ABn(1+n)^2(n+2)^2}{(2n+3)^3} > F_{Outside} \quad (8)$$

Therefore, mutualism is always more easily favoured with host rewarding than without. In addition, when hosts reward symbiont cooperation mutualism always becomes more likely as the number of mutualists increases (Figure 3). The reason for this that host fitness benefits from symbiosis increase, while a tragedy of the commons is averted because through host rewarding cooperating symbionts preferentially receive host benefits, maintaining cooperation even with high symbiont numbers (Figure S1). Thus, host rewarding of cooperative symbionts may help enable the high symbiont numbers required to enable division of labour among symbionts (result 2).

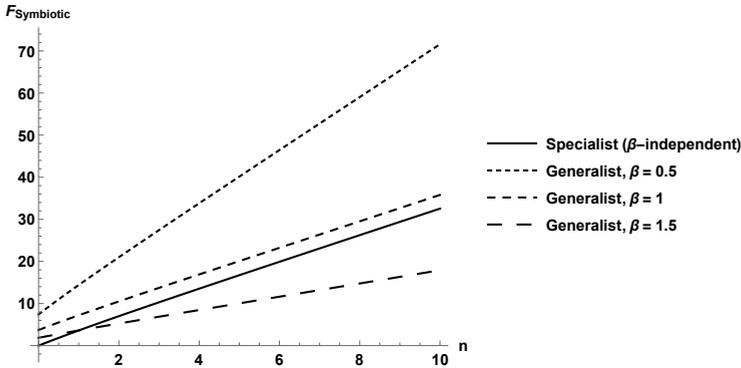


Figure 3: Symbiont cooperation conditions in the presence of host rewarding (A=10, B=10).

If hosts preferentially reward cooperative symbionts, symbiont cooperation is always more easily maintained with increasing symbiont numbers. Again, cooperation is more easily maintained in generalist symbionts than in specialised ones, except under antagonistic resource transfer ($\beta > 1$).

Host choice of symbiont type stabilises mutualism and division of labour

Lastly, we consider a scenario where, if there is a symbiotic division of labour (*i.e.* if inequality (2) is met), hosts are able to actively choose symbiont types from the environment. We were interested in this scenario, because of our finding that in the case of symbiont specialisation mutualism is difficult, particularly at low symbiont numbers (Figure 2 & 3). We therefore modelled a scenario where hosts could increase their chance of selecting a symbiont community containing both specialist types. We assumed that this partner choice was not perfect, but rather that its strength would be determined by parameter s . We only modelled the effect of partner choice for specialised

symbionts, because if there is no division of labour among symbionts there is only a single symbiont type and hosts cannot make a choice.

We found that partner choice by hosts increases the domain over which mutualism is a favourable option for symbionts, particularly at low symbiont numbers (Figure 4, Appendix Equations A17 & A18). The reason for this is that partner choice increases the likelihood of a balanced symbiont population, increasing host fitness and increasing the chance that investing in the host is favourable to symbionts. Partner choice is more effective with stronger host preference (*i.e.* higher s). Importantly, host choice for symbiont type can facilitate mutualism both in the absence (Figure 4A) and presence of host rewarding of symbiont cooperation (Figure 4B), with mutualism being easiest to maintain when both are present. This shows that the two active host strategies we have considered, hosts *choosing* partner type and hosts *rewarding* partner cooperation, can work together to maintain mutualism and enable stable symbiotic division of labour.

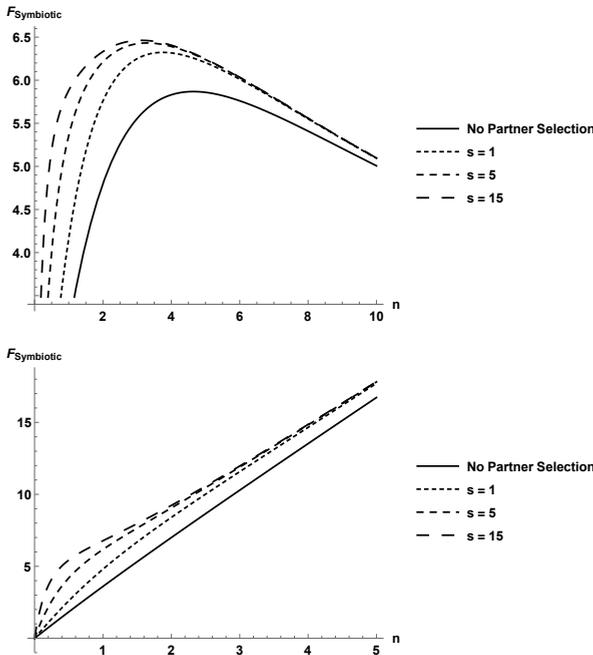


Figure 4: Symbiont cooperation conditions when there is potential partner selection of symbionts by the host, for various levels of partner preference strength (s).

The top panel shows a scenario without host rewarding of symbiont cooperation, while the bottom panel shows a scenario where hosts reward symbiont cooperation with additional resources. The continuous lines represent a case without partner selection. These are the same as those in respectively Figure 2 and 3, and are printed to facilitate comparison.

Discussion

Many hosts interact with multiple mutualists simultaneously, rather than with only a single one. Often, these hosts obtain a range of different services and resources symbiotically, from protection and pollination to nutrients. These two observations combined mean there is potential variation in the overlap between mutualists in terms of services provided (Afkhani *et al.*, 2014) and that there is the potential for a division of labour, where each mutualist specialises on a single service or resource. If there is such a division of labour, we expect multiple mutualists to have synergistic effects, reinforcing each other's positive effect on host fitness (Afkhani *et al.*, 2014). While such synergism between multiple mutualist was observed repeatedly (Stachowicz & Whitlatch, 2005; McKeon *et al.*, 2012; Fraune *et al.*, 2015; van der Heijden *et al.*, 2015) and while many models have asked when mutualistic cooperation with a host is favoured (*e.g.* Schwartz & Hoeksema, 1998; West *et al.*, 2002b; Foster & Kokko, 2006), we did not have a formal general model of when mutualist cooperation is expected to be generalist in nature (*i.e.* when mutualists provide multiple benefits) and when mutualists will specialise on providing one benefit.

We generated such models and show that specialisation is favoured if there is antagonism between the resources provided and if there are high symbiont numbers per host (inequality (2)). However, we also find that mutualism is most easily maintained at intermediate symbiont numbers (inequalities (4) and (5), Figure 2). Therefore, it may be difficult to achieve the high symbiont numbers required to enable symbiont specialisation. We explore two host strategies to solve this challenge. First, host preferential rewarding of cooperative symbiont behaviour prevents mutualism collapse and can thus enable the evolution of symbiont specialisation (inequalities (7) and (8), Figures 3 and S1). Second, active host choice of diverse symbiont communities stabilises specialised mutualisms at low symbiont numbers and increases the domain over which a symbiotic division of labour and stable mutualism can coexist (Figure 4). These results show that the evolution of the optimal *level* and of the *nature* of mutualistic cooperation are related, and that both can be affected by host strategies. Thus, our results help understand both *if* symbionts should cooperate with hosts (*i.e.* level of investment in cooperation), but also *how* they should cooperate (*i.e.* what they should provide).

Trade-offs are key in symbiotic divisions of labour

Our results show that the first key factor driving division of labour among symbionts is trade-off strength, or β (inequality (2)). Trade-offs can also drive the evolution of habitat and resource specialisation (*e.g.* Day, 2000; Egas *et al.*, 2004; Parvinen & Egas, 2004; Débarre & Gandon, 2010; Raymond & Bonsall, 2013; Østman *et al.*, 2014), division of labour within organisms (Michod, 2006; Rueffler *et al.*, 2012; Goldsby *et al.*,

2014) and potentially even biochemical division of labour between genes and enzymes (Boza *et al.*, 2014). In the context of mutualistic resource transmission, we find that antagonistic resource transmission (or strong trade-offs, $\beta > 1$) favours specialisation and complementary resource transmission favours (or weak trade-offs, $0 < \beta < 1$) symbiont generalism (Figure 1).

This raises the biological question when resource transmission is antagonistic and when is it complementary? We expect this to be highly mutualism-specific. If two resources require very different adaptations to obtain or provide, this will drive strong trade-offs. Do the two resources require separate and energetically costly pathways for uptake, production or metabolism, or can a single pathway be co-opted? Does a symbiont need to invest in a separate structure, or can a second resource be processed as a by-product (Hoeksema & Schwartz, 2003)?

Obtaining soil resources as a by-product may explain why arbuscular mycorrhizal fungi (AMF) often function as generalist, multifunctional symbionts (Newsham *et al.*, 1995; Sikes *et al.*, 2010). The main benefits of AMF to plant hosts, providing phosphorus (Parniske, 2008), nitrogen (Leigh *et al.*, 2009; Fellbaum *et al.*, 2012) and drought resistance (Augé, 2001) are probably a function of hyphal network size in the soil (Avio *et al.*, 2006). An AMF's investment in hyphae to extract more phosphorus will also enable retention of extra water and acquisition of additional nitrogen (although at the molecular level, investment in nitrogen transporters might to some extent come at the expense of phosphorus transporters). Consequently these AMF services to the plant will be complementary rather than antagonistic (*i.e.* $\beta < 1$), favouring generalist cooperation. This will favour AMF to provide generalist services, even though other characteristics of the mutualism like large numbers of symbionts (fungi) per host (Davison *et al.*, 2011) and preferential rewarding to stabilise fungal cooperation (Bever *et al.*, 2009; Kiers *et al.*, 2011), would enable divisions of labour. In contrast, AMF protection from pathogens is thought to require large hyphal networks within the roots (Newsham *et al.*, 1995), which may trade-off with soil network size. This could be a reason why some AMF are better pathogen suppressers than nutrient providers (Klironomos, 2000; Sikes *et al.*, 2010; Chagnon *et al.*, 2013) and highlights that trade-off strength (β) depends on the services considered. For AMF, low β might exist between phosphorus and nitrogen provision, while there is a high β between phosphorus and pathogen protection.

Generalist symbionts may offer multiple services as by-products of their association with a host. For example, anemones benefit from protection provided by clownfish and also from the ammonium they secrete as waste products (Roopin & Chadwick, 2009), which presumably comes at no cost to these fish. *Hamiltonella defensa* defensive aphid symbionts can protect against phylogenetically distant parasitoid wasps (albeit

with variable effectiveness), probably because the toxins targeting one clade of wasps are also (somewhat) effective against other wasps (McLean & Godfray, 2015). In contrast, specialist symbionts are more likely when two different mutualistic services require fundamentally different apparatus. A perhaps trivial example is plant pollination and mineral acquisition: because of the physical separation of roots and flowers, as well as the motility required of pollinators, the trade-off between these services is very strong. Hence, there is a division of labour between bees and symbiotic root microbes, and there are to our knowledge no mutualists that can both pollinate plants and provide soil nutrients (although a bat species has been described that fertilises carnivorous pitcher plants with its nitrogen-rich faeces (Grafe *et al.*, 2011; Schöner *et al.*, 2015)).

Similarly, the decomposition of wood requires a range of costly enzymes: consequentially, there is a division of labour among symbionts of wood-decomposing termites, where termites rely on fungi to break down plant substrate before ingestion and then on bacteria to digest the resulting simpler oligosaccharides (Poulsen *et al.*, 2014). However, strong inherent trade-offs are not necessarily required for specialisation, as suggested by our model (inequality (2)). In some cicada species, bacterial endosymbionts have split into two different specialised species fulfilling different functions (Van Leuven *et al.*, 2014). The fact that these functions were previously performed by a single species suggests that trade-offs cannot have always made mutualistic generalism insurmountably costly. Probably, a single bacterial endosymbiont, providing multiple functions, was originally acquired by the cicada host, only later to split in two separate lineages when higher symbiont population sizes were achieved (Van Leuven *et al.*, 2014). Hence, trade-offs are important predictors of symbiotic division of labour, but not the only factor. Our results show that a second important key factor is the number of symbionts per host (inequality (2)).

A tragedy-of-the-commons can prevent symbiotic divisions of labour

Symbiont specialisation and random uptake of symbionts from the environment comes with the risk of missing a crucial mutualistic resource or service. Our results indicate larger numbers of symbionts per host favour symbiont specialisation, by increasing the probability the host has access to both types. Maintaining high symbiont numbers can thus be considered a host 'insurance' against missing certain resources when symbionts are specialised (Yachi & Loreau, 1999). Larger colony or population size can also favour the evolution of division of labour in organisms as diverse as algae (Solarì *et al.*, 2006; Østman *et al.*, 2014; Ferguson-Gow *et al.*, 2014). Yet, for symbionts interacting with a host, high symbiont numbers can cause a breakdown of mutualism because they drive conflict among different symbionts (Frank, 1997; Poulsen & Currie, 2010; Friesen & Mathias, 2010), which then exploit their host and rely on other symbionts to cooperate, a tragedy-of-the-commons (Rankin *et al.*, 2007). The problem of mutualistic interactions

turning less beneficial or even detrimental to the host with increasing symbiont numbers has indeed been found in some mutualisms. For instance, worms that clean material from the filaments of crayfish gills become less beneficial as their numbers increase (Brown *et al.*, 2012). Similarly, the relationship between flowers and pollinating bees can shift from mutualistic to antagonistic when bees become very abundant (Aizen *et al.*, 2014). Our model also predicts this effect (inequalities (4) and (5), Figure 2). Therefore, the conditions under which symbiont specialisation is more likely to be favoured are also the conditions under which the mutualism is more likely to break down. Furthermore, our model predicts that mutualism is usually more easily maintained with generalist symbionts because these symbionts do not need to rely on the traits of other symbionts to receive return benefits from their hosts (Figure 2), but otherwise the tragedy-of-the-commons at high partner numbers makes the evolution of specialisation in mutualisms unlikely. So, why do we in fact observe specialised and still beneficial mutualisms?

Host strategies modifying symbiont traits and communities enable specialisation

We explored two strategies host might employ with the potential to solve this puzzle. First, hosts could evolve mechanisms that favour high levels of cooperation in symbionts, which in turn could increase the range of conditions in which stable symbiont division of labour and stable cooperation coexist. Second, if a division of labour arose, hosts could evolve mechanisms to favour balanced symbiont communities, remedying the problem of lacking a key resource. Regardless of symbiont specialisation, hosts should prefer mutualists that cooperate more: they are favoured to acquire traits that increase symbiont cooperation. One set of strategies that hosts can adopt to favour higher symbiont cooperation and resolve the tragedy-of-the-commons is preferential rewarding of cooperative symbionts, or the sanctioning of less cooperative symbionts (Noë & Hammerstein, 1994; Schwartz & Hoeksema, 1998; West *et al.*, 2002b; Werner & Kiers, 2015a; Chapter 3). This happens in many mutualisms. For instance, *Yucca* plants abort fruits when the moths that pollinate their flowers deposit too many eggs in the fruit (Pellmyr & Huth, 1994). Similarly, aphid hosts provide purines only to *Buchnera* bacteria synthesising the amino acid histidine for their hosts (Thomas *et al.*, 2009; Price *et al.*, 2014), ‘client’ fish sanction low-quality ‘cleaner fish’ (Bshary & Grutter, 2005) and plants preferentially allocate photosynthate to symbiotic root nodules providing nitrogen (Kiers *et al.*, 2003). We studied the effects of a relative rewarding scheme where hosts reward symbionts proportionally to their level of cooperation (*i.e.* investment in the host) (Wyatt *et al.*, 2014). Alternatively, this strategy can be thought of as a strategy of flexible sanctions for uncooperative (relative to the average population level) symbionts (West *et al.*, 2002b). We show that host rewarding stabilises symbiont cooperation even when there is a large number of symbionts (equations (6), Figure S1). Both for symbiont generalism and specialisation, host rewarding increases the conditions over which symbionts prefer a mutualistic lifestyle over a non-cooperative one (equations (7)

and (8), Figure 3). This means that host rewarding of cooperation in potential partners can both facilitate the high symbiont numbers at which symbiont specialisation evolves from generalism more easily, as well as expanding the domain over which a symbiotic division of labour can coexist with symbiont investment in the host.

Whilst hosts prefer higher cooperativeness, it is unclear whether or not hosts prefer symbiont specialisation. They clearly prefer a mutualistic system that is more efficient overall, so would favour specialisation if there are strong trade-offs (equality 2), but hosts also run the risk of being left without one of the resources. This is the same evolutionary problem that symbionts face. However, we know that in many mutualisms hosts can detect symbiont identity, for instance through signalling, and exert some influence (choice) over the identity of their partners (Bright & Bulgheresi, 2010). For instance, this happens in the association between plants and rhizobial bacteria through extensive communication before the rhizobia enter nodules (Oldroyd, 2013) and similarly in the association of squid and bioluminescent bacteria (McFall-Ngai *et al.*, 2012; Kremer *et al.*, 2014; Heath-Heckman *et al.*, 2014). It also happens when ants select single spores of fungi that they grow for food to inoculate new colonies (Aanen *et al.*, 2009). We found that, when there is division of labour, if hosts can detect symbiont type and favour a more equally balanced community (albeit imperfectly), mutualism is more easy to maintain (Figure 4, Top). The reason for this is that such host choice reduces the risk of being left without a key resource, meaning that investment in the host is more likely to be worthwhile for symbionts. This indicates that, other than host rewarding of symbiont cooperation, host choice of symbiont type can also expand the conditions where symbiotic division of labour can stably exist. Mutualism is easiest to maintain if hosts employ both rewarding and partner choice simultaneously (Figure 4, Top), indicating that both strategies can interact to synergistically favour conditions for specialised mutualisms to evolve.

Open questions

While our current models provide considerably insight into factors driving the evolution and stability of a division of labour among a mutualist community, many questions remain. For instance, our models do not consider spatial structure (Débarre & Gandon, 2010), but rather implicitly assume symbionts and hosts are well-mixed. In host-parasite interactions, spatial structure eases the evolution of generalist parasites (Ashby *et al.*, 2014). Spatial structure can also be important in mutualism evolution, because it can locally limit the availability of symbiotic partners (Verbruggen *et al.*, 2012). If spatial structure more generally limits the local availability of diverse symbiont types, we expect this could impede the evolution of symbiont specialisation by limiting the host's ability to obtain all symbiont types required to meet its needs.

This suggests the idea that specialised mutualisms, because hosts requires on the continued presence of more symbiont types, may be more vulnerable to the (temporary or spatial) absence of a given symbiont, particularly if the presence of a symbiont species is unreliable (Palmer *et al.*, 2003). It has been argued that obligate mutualisms are more likely to suffer from mutualism breakdown and extinction than facultative interactions, because they are less robust to (temporary) absences of one partner (Sachs & Simms, 2006). A similar reasoning may be applied to specialised versus generalist mutualisms: a division of labour could make mutualisms more susceptible to variation the density of crucial mutualistic partners. However, the effect of volatile (environmental) conditions, such as variable partner or resource densities, on the evolution of a division of labour among symbionts has not yet been explored. It has been suggested that symbiont generalism can serve as strategy to ensure a symbiont's value to a host in a range of conditions (Werner *et al.*, 2014c; Chapter 2). In general, specialisation (for instance on habitats or resources) is thought to be more likely to evolve in stable than in variable environments (Condon *et al.*, 2014; De León *et al.*, 2014). Future research can now address the effect of spatial and temporal volatility in factors such as host, symbiont and resource abundance on the evolution of symbiont specialisation.

We have assumed that the host can only obtain the two resources through mutualism. While in many cases this may be biologically plausible, in some interactions mutualistic services may be (partially) replaceable by non-symbiotic means. For instance, most plants obtain a substantial proportion of soil nutrients directly through their roots and use soil mutualists to increase their nutrient supply (Parniske, 2008). Wind-pollination can similarly serve as an alternative to mutualistic pollination. Therefore, it would be interesting to expand our models to include potential non-symbiotic ways of obtaining resources and services. Briefly, we would expect that if a host can obtain certain services non-symbiotically, symbiotic specialisation may be easier to evolve because there is a lower risk of missing that service. However, such a model would need to consider the consequences this would have for the host's energy budget, including trade-offs with other crucial functions. If these are strong, mutualistic exchange may still be favoured even when directly obtaining the services is possible in principle (Schwartz & Hoeksema, 1998; Hoeksema & Schwartz, 2003).

Another important aspect is that, once a symbiont has specialised on a single service, a return to generalism may be more difficult to evolve than the original transition. The reason for this is that divisions of labour, and the loss of traits because they are provided by biotic partners, will often be driven by loss of the genes regulating these traits (Visser *et al.*, 2010; Ellers *et al.*, 2012; Bennett & Moran, 2013; Pande *et al.*, 2014). As a consequence, specialising on a single service and thereby becoming reliant on other symbionts, may represent an evolutionary 'rabbit hole', similar to relying on

endosymbionts from the host perspectives (Bennett & Moran, 2015) or the risk of specialising on a single host as an evolutionary dead-end (Tripp & Manos, 2008). In our models, we found that the initial condition is one of a single generalist symbiont from which specialisation evolves. While this is consistent with a scenario where the transition from generalism to specialisation is easier than the reverse, we do not explicitly model the genetic basis for our specialisation trait (α) and therefore do not directly study a potential bias towards specialisation.

Lastly, a key challenge in applying our framework to actual mutualisms, both experimentally and in the field, will be how to precisely define the mutualistic services or resources studied, and measure the trade-off between them. As we have seen, many hosts receive multiple mutualistic services, with potentially different trade-off curves, and measuring these is both challenging and labour intensive. Yet, to further our understanding of the evolutionary pressures symbionts face, trade-offs need to be measured rather than only assumed (Wilson & Yoshimura, 1994; Asplen *et al.*, 2012). For instance, insectivorous bats are actually more efficient pollinators than specialised nectar bats (Frick *et al.*, 2013), highlighting that expected advantages of specialisation may not always arise in the field.

Conclusions and predictions

A division of labour among symbionts specialising in different mutualistic services or resources to the host can be difficult to evolve. It requires strong trade-offs and high symbiont numbers. We identify host rewarding of cooperation and active partner choice by the host as factors that can facilitate the evolution and stability of specialisation. In the absence of these factors symbiont specialisation is only expected to evolve at low to intermediate numbers of symbionts and with strong trade-offs. To our knowledge, this is the first work to study the conditions for a division of labour among different symbionts evolving. This question fits in the general framework of Multiple Mutualist Effects (MME) to study the effect of multiple mutualists on mutualist and host dynamics (Afkhani *et al.*, 2014). Their notion of overlap in mutualistic rewards is closely related to what we called a division of labour among symbionts. Highly specialised, non-overlapping symbionts should have synergistic fitness effects on hosts (Afkhani *et al.*, 2014). Indeed, a recent meta-analysis of MME in plant mutualisms revealed that there are often such synergistic effects but that variability is very large (Morris *et al.*, 2007), suggesting symbiotic division of labour is common but not universal among mutualisms. Further analysis of the importance and occurrence of divisions of labour among symbionts will now require measuring complementarity and synergism between different symbionts across various mutualisms. Do hosts with more symbiotic partners also harbour more and stronger specialised symbiont types? Based on our analyses, we predict that (in a meta-analysis) mutualistic synergism and number of distinct symbiont

types positively correlate with symbiont numbers per host, and with trade-off strength between the services provided.

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