

LETTER

Why mutualist partners vary in quality: mutation–selection balance and incentives to cheat in the fig tree–fig wasp mutualism

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Abstract

Mutualisms between species are ecologically ubiquitous but evolutionarily puzzling. Host discrimination mechanisms that reduce the fitness of uncooperative symbionts can stabilise mutualism against collapse, but also present a paradox – if discrimination is effective, why do uncooperative symbionts persist? Here, we test whether mutations or fitness benefits of cheating best explain the prevalence of uncooperative wasps in the fig tree–fig wasp mutualism. By combining theory with field-collected data we demonstrate that the proportions of pollen-free wasps of strongly discriminating hosts are reached with reasonable mutation rates. In contrast, in weakly discriminating hosts, the required mutation rates, assuming a single locus, are untenably high, but the required cheater advantages fall within expected ranges. We propose that when discrimination is weak, uncooperative symbionts proliferate until they reach the equilibrium proportion that balances costs and benefits of cheating. Our results suggest that mechanisms that resolve the paradox of uncooperative symbionts differ among host species.

Keywords

Conflict, cooperation, host sanctions, mutualism, partner choice.

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INTRODUCTION

Mutually beneficial interactions between species (mutualisms) are essential for many ecosystems. For example, mycorrhizal fungi provide nutrients to forest trees, pollinators help flowering plants set fruit, and intestinal bacteria help animals take up nutrients (Herre *et al.* 1999; Ollerton *et al.* 2011; Rakoff-Nahoum *et al.* 2016). In most mutualisms, hosts encounter symbionts of varying quality, spanning highly cooperative symbionts that provide hosts with services they cannot perform themselves to uncooperative symbionts that provide no services (Bronstein 2001; Sachs & Simms 2006; McNamara & Leimar 2010). To ensure the highest return on investment, discriminating hosts in several mutualisms allocate more resources to tissues supporting highly cooperative symbionts (Kiers *et al.* 2003, 2011; Bever *et al.* 2009; Bever 2015; Jandér & Herre 2016). In the absence of counter-balancing mechanisms, such host discrimination can eliminate uncooperative symbionts from mixed populations (Heath & Stinchcombe 2013). However, in empirical systems researchers find persisting variability in the levels of symbiont cooperation (Heath & Tiffin 2007; Jandér & Herre 2010; Sachs *et al.* 2010). The purpose of this study is to determine which counterbalancing mechanisms maintain uncooperative symbionts in the mutualism between fig trees and pollinating fig wasps.

Two distinct mechanisms can sustain uncooperative symbionts in the face of host discrimination. First, in mutation–selection models, random mutations can result in loss of symbiotic function. When mutation rates reach equilibrium with the

selection against uncooperative symbionts caused by host discrimination, cooperative and uncooperative symbionts can coexist (Foster & Kokko 2006). Second, in cost–benefit models, uncooperative symbionts benefit by shifting resources (such as energy or time) away from providing symbiotic resources and into promoting their own fitness (Denison 2000; West *et al.* 2002). While hosts can use discrimination mechanisms to prevent uncooperative symbionts from collapsing the mutualism, limitations in the precision of discrimination can allow cheaters to coexist with mutualists (Friesen & Mathias 2010; Jandér *et al.* 2012; Steidinger & Bever 2016). The two models differ primarily in the fitness that uncooperative symbionts receive in the *absence* of host discrimination: in mutation–selection models, cooperative and uncooperative symbionts have equal fitness, whereas in cost–benefit models uncooperative symbionts have relatively higher fitness because they do not expend resources on costly cooperation with their host, and are therefore true ‘cheaters’ (Ghoul *et al.* 2014; contrast with definition in Jones *et al.* 2015).

The fig tree–fig wasp mutualism presents an unusual opportunity to study this question. Fig wasps are the sole pollinators of their fig species, and fig wasp offspring can only develop in galled fig flowers (Herre *et al.* 2008). Actively pollinating fig wasps collect pollen with their front legs and store it in specialised pollen pockets (Frank 1984). While most wasp individuals carry pollen, some do not – their pollen pockets are empty and they can therefore not pollinate their host. Such pollen-free, uncooperative, wasps occur in all four actively pollinating fig wasp species surveyed so far in Panama

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(0.3–5% depending on species, Jandér & Herre 2010). The corresponding fig tree species have discriminative mechanisms (termed host sanctions; a type of partner choice) that lower the fitness of wasps that do not pollinate: trees are more likely to abort unpollinated figs (thereby killing the wasp larvae within), and wasp offspring that develop in unpollinated figs are fewer and smaller than those that develop in pollinated figs (Jandér & Herre 2010; Jandér *et al.* 2016). Host sanctions vary in strength across fig species, and are likely due to selective resource allocation by the host to better pollinated figs (Jandér & Herre 2010, 2016).

Here, we use mathematical models parameterised with empirical data from four fig species and their respective wasps to determine the mechanisms likely to maintain observed proportions of pollen-free wasps of the pollinator species. First, we calculate the fitness costs caused by sanctions for an average pollen-free wasp of each species. We then determine what mutation rate (under the mutation–selection model) or relative fitness benefit (under the cost–benefit balance model) would be required to explain the proportion of pollen-free wasps that we encounter in the field. We find that for strongly sanctioning host species the observed proportion pollen-free wasps is reached with reasonable mutation rates. In contrast, in weakly sanctioning host species the required mutation rates are untenably high but the required cheater benefits fall within expected ranges. The mechanisms that resolve the paradox of uncooperative symbionts therefore likely differ among host–symbiont pairs.

METHODS

Study system

Fig trees and their pollinating wasps are a keystone mutualism for tropical forests (Shanahan *et al.* 2001). Each of the over 800 known fig species typically interacts with only one or two pollinating wasp species (Molbo *et al.* 2003; Cruaud *et al.* 2012). All empirical data in this study originate from natural fig and wasp populations at the Barro Colorado Nature Monument (BCNM), Panama, with species characteristics in Table S1 (Supporting Information); all fig species in this study are monoecious and actively pollinated. *Ficus citrifolia* is here pollinated by *Pegoscapus tonduzi*, *F. nymphaeifolia* by *P. piceipes*, *F. obtusifolia* by *P. hoffmeyerii* A and *P. hoffmeyerii* B, and *F. popenoei* by *P. gemellus* A and *P. gemellus* B (Wiebes 1995; Molbo *et al.* 2003). For simplicity we will use the names of the fig species also when referring to the associated wasp species, e.g. *F. popenoei* A refers to *P. gemellus* A.

Fig trees produce hundreds of flowers within each hollow inflorescence ('fig'). One or several female wasps ('foundresses') enter each fig and pollinate and lay eggs in the flowers; each flower can develop into either a seed or feed a single wasp. Male wasps emerge first, mate with the females and chew an exit tunnel. Females of actively pollinating species use their front legs to collect pollen, store it in thoracic pollen pockets, and once at a receptive fig, deposit the pollen using their front legs (Frank 1984; Jandér & Herre 2010). Wasps can only collect pollen from their natal fig, never later. Female wasps in search of a flowering tree often disperse long distances, at BCNM on average 10 km (Nason *et al.* 1998),

despite only being a few millimetres long and having an adult lifespan of 1–3 days (Dunn *et al.* 2008; Jevanandam *et al.* 2013; Jandér unpublished data). Dispersal is perilous; less than 1% of female wasps make it to a flowering fig (Herre 1989; Jandér *et al.* 2016). Factors that increase the probability of successful dispersal therefore directly increase wasp fitness. Importantly, lifetime reproductive success of female fig wasps in the field is easily quantified (Herre 1989; Jandér & Herre 2010). We have previously quantified the fitness effects of not pollinating for single foundresses of the studied wasp species using manipulative field experiments (Jandér & Herre 2010).

GENERAL MODEL PARAMETERISED TO THE FIG WASP MUTUALISM

First, we calculate the fitness costs (due to host sanctions) of not pollinating, ignoring any potential benefits. The costs for a non-pollinating (P–) wasp of the pollinating species depend both on the strength of sanctions and on the likelihood of sharing a fig with other P– wasps (because P– wasps may free-ride on the pollination efforts of pollen-carrying (P+) foundresses with which they share fig; Jandér *et al.* 2012). We assume: (1) If there are multiple foundresses in a fig, offspring are divided equally among them (supported by Jandér *et al.* 2012). (2) Foundresses in a fig are unrelated. This is reasonable given the distances that wasps disperse (Nason *et al.* 1998). (3) Wasps within the same pollinator type (e.g. P–) have equal oviposition rates, but, as we see later, the P– rate may differ from the rate of P+ wasps. We first modify the general model for the fitness of cooperative and uncooperative symbionts that associate with a host that discriminates among the services generated in discrete modules (Steidinger & Bever 2016) to better fit the fig system (Jandér 2011). Step by step derivations of all of the terms, including mathematical code, are provided in the online appendix, and notations are listed in Table 1.

Let the fitness of P+ wasps be determined by eqn 1, such that

$$W_{P+} = \sum_{N=1}^{N_{\max}} p_N \sum_{Q=0}^{Q=N} \overbrace{\left[\frac{N-Q}{1-qN} \right] \left[\left(\frac{Q}{N} \right) q^Q (1-q)^{N-Q} \right]}^A \underbrace{\left[\frac{K(1 - e^{-r(N+Q(w-1))/K})}{N+Q(w-1)} \right]}_B \underbrace{\left[\phi + (1-\phi) \left(\frac{\alpha(N-Q)}{N\alpha + Q(2\alpha-1)} \right) \right]}_C \quad (1)$$

and the fitness of P– wasps by eqn 2

$$W_{P-} = wk \sum_{N=1}^{N_{\max}} p_N \sum_{Q=0}^{Q=N} \overbrace{\left[\frac{Q}{qN} \right] \left[\left(\frac{Q}{N} \right) q^Q (1-q)^{N-Q} \right]}^A \underbrace{\left[\frac{K(1 - e^{-r(N+Q(w-1))/K})}{N+Q(w-1)} \right]}_B \underbrace{\left[\phi + (1-\phi) \left(\frac{\alpha(N-Q)}{N\alpha + Q(2\alpha-1)} \right) \right]}_C \quad (2)$$

Beginning with eqn 1, let p_N be the probability a wasp ends up in a fig with N other foundresses. To calculate p_N , we

Table 1 Summary of the main notations

Notation	Value	Description
F	Field	Inbreeding coefficient ($0 \leq F \leq 1$); F_A and F_B measured from molecular markers for wasp species A and B, whereas F_{H1} and F_{H2} are estimated from the harmonic mean foundress numbers (H) from two datasets (1 and 2)
k	Calc	Relative likelihood of arrival at a flowering tree for a P– wasp compared to a P+ wasp
K	Field	Species-specific maximum number of wasp offspring that can emerge from a fig
N	Variable	Number of foundresses in a fig ($N = 1, 2, 3, \dots, N_{max}$)
N_{max}	Field	Maximum recorded number of foundresses in a fig ($P_N > N_{max} = 0$)
N_e	Calc	The effective population size of each wasp species
P_N	Field	Proportion of figs with N foundresses ($\Sigma P_N = 1$)
Q	Variable	Number of P– wasps in a fig ($Q = 0, 1, 2, 3, \dots, N$)
q	Variable	Proportion of P– wasps in the population ($0 \leq q \leq 1$)
q_{field}	Field	Proportion of P– wasps in natural populations, from field collections
q_{mut}	Calc	Proportion of P– wasps in the population, calculated for different mutation rates
r	Calc	Species-specific oviposition rate of foundresses
s	Calc	Selective disadvantage for a P– wasp compared to a P+ wasp (selection coefficient, $0 \leq s \leq 1$)
μ	Calc and variable	Mutation rate per locus per generation. The mutation rate required to account for q_{field} is calculated for each fig wasp species. When q_{mut} is calculated, μ is a variable that has values 10^{-6} , 10^{-5} , 10^{-4} and 2.84×10^{-5}
w	Calc	Relative oviposition rate of a P– wasp compared to a P+ wasp
$W_{P-}; W_{P+}$	Calc	Fitness (expected number of offspring) for a P– or P+ wasp
α		Half-saturation constant determining how much resources are allocated to a fig with a 50 : 50 mix of P– and P+ foundresses ($0.5 \geq \alpha < 1$)
ϕ	Field	The proportion of offspring that develop in figs colonised by only P– wasps compared to figs colonised by only P+ wasps

Values are indicated as ‘field’ for field collected (exact values; listed in Tables S2–S4 where previously unpublished), ‘calc’ for calculated using the models presented here parameterised with field-collected data, or variable for general parameters.

determined the proportion of figs with N foundresses in the field (Jandér & Herre 2010; Appendix). Thus, the $\Sigma p_N ABC$ is the sum of the product of the three other terms that include N weighted by their probability.

Briefly, A uses the binomial probability function to determine the probability that a fig containing N wasp foundresses will contain Q P– wasps, B determines the wasp fitness before host discrimination given that P+ wasps oviposit at rate r and P– wasps oviposit at rate rw into figs with a carrying capacity of K (the maximum number of wasp offspring a single fig can produce; Herre 1988, 1989), and C determines the effects of host discrimination on wasp fitness.

For the term C , ϕ is the proportion of the total fitness (given by B) that a wasp would get if it occurs in a fig where all the foundresses are P– ($Q = N$). The value of ϕ for each species was calculated from field experiments documenting the effect of host sanctions in single-foundress figs (Jandér & Herre 2010). The product

$$(1 - \phi) \left(\frac{\alpha(N - Q)}{N\alpha + Q(2\alpha - 1)} \right)$$

is the extra proportion of B offspring that wasps have in mixed figs (that contain both P– and P+ wasps) due to partial pollination (Jandér *et al.* 2012). The term α is the half-saturation constant, such that $C = \phi + (1 - \phi)\alpha$ proportion of B offspring is derived from a wasp in a fig with a 50 : 50 mix of P+ and P– wasps (where $Q = N/2$) (Steidinger & Bever 2016).

For both mutation–selection and cost–benefit models, we will consider host discrimination along a range of half-saturation constants from $\alpha = 1/2$ to $\alpha \approx 1$. Lower values of α result in harsher host discrimination against mixed figs. For

example, when $\alpha \approx 1$, the presence of a single pollinator in a fig is sufficient to mitigate sanctions for all foundresses in that fig. In this special case, term C simplifies to a step function (Fig. S2), such that:

$$C|_{\alpha \approx 1} \begin{cases} 1 & (N - Q) \geq 1 \\ \phi & (N - Q) = 0 \end{cases}$$

By contrast, at lower values of α , figs will apply intermediate sanctions on mixed figs that contain both P+ and P– wasps. When $\alpha = 0.5$ the amount of resources that the fig receives are linearly dependent on the proportion of foundresses that pollinate it (Fig. S2). Available empirical evidence supports an α close to 1 for these species (Jandér & Herre 2010, 2016; Jandér *et al.* 2012, 2016). We therefore focus on the results from a model based on $\alpha \approx 1$, but will for comparison also present results for other values of α .

The fitness of P– wasps (eqn 2) also includes the terms A , B and C , however, the equations differ in two ways: (1) the A term for the P– wasps has a different weighting term (Q/Nq); (2) the equation includes the product wk , which is product of the relative oviposition rate of P– wasps and the relative likelihood that P– wasps (compared to P+ wasps) will arrive at a receptive fig in which to lay their eggs. The first difference is necessary as the probability that a P+ and P– wasp end up in a fig with Q P– wasps is usually different. The second point includes the terms that determine whether or not a P– wasp is a cheater. P– wasps are cheaters (i.e. benefit from not pollinating) if, relative to P+ wasps, they have either higher oviposition rates ($w > 1$), or are more likely to encounter a receptive fig ($k > 1$). In the mutation–selection model where P– wasps do not have benefits over P+ wasps, we set $w = k = 1$.

Results

The extent to which host sanctions select against P– wasps is quantified by the coefficient of selection, s , which can be calculated for each fig species. We set $W_{P+} = 1$ and $W_{P-} = 1 - s$, such that $s = (W_{P+} - W_{P-})/W_{P+}$. While the strength of selection against P– wasps differs across the four fig species, W_{P-} is always negatively frequency dependent (Fig. 1, Fig. S4). This is because P– wasps are less likely to avoid sanctions by free-riding on the pollination efforts of P+ wasps when P– wasps increase in relative frequency. Selection against P– wasps that are rare (the field-based q , q_{field} , is ≤ 0.05 for all P– wasps studied here) is highest when partner choice is linear, and weakest when $\alpha \approx 1$ (Fig. 1).

MUTATION–SELECTION MODEL FOR BEHAVIOURAL POLYMORPHISM

In the mutation–selection model, the behavioural polymorphism is stable when selection removes P– wasps from the population at the same rate that mutation reintroduces them. We determined the necessary mutation rate to sustain P– wasps at their observed frequencies using an equilibrium model (Falconer & Mackay 1996) modified for haplodiploid populations where selection acts only on females (Crozier

1976; Werren 1993; see Appendix). We assumed: (1) the pollen-free trait is heritable (supported by preliminary data; see Appendix), (2) migration and drift are unimportant (populations are very large; see Appendix) and (3) that pollination behaviour is determined by one locus with a dominant allele A, and a recessive allele a; such that female P+ wasps have the alleles AA or Aa, and P– wasps have the fully recessive alleles aa. This model is a conservative estimate of the proportion of P– wasps that can be explained by mutation rates alone. Alternative models where pollination is determined by multiple loci and mutations at any one of them could disrupt pollination behaviour would require lower mutation rates to explain the observed levels of P– wasps (Van Dyken *et al.* 2011); see Appendix.

For a recessive allele in a haplodiploid species (males haploid) where the selection acts only on females, and with the inbreeding coefficient F , the mutation rate (μ) per generation (that converts allele A to a) required to sustain P– wasps at the observed proportion q_{field} is equal to (eqn 3)

$$\mu = \frac{(-F + \sqrt{F^2 + 4q_{field}(1-F)})^2 s}{6(1-F)^2}, \quad (3)$$

where s is the selective disadvantage incurred by homozygous females (calculated from field data in the previous section).

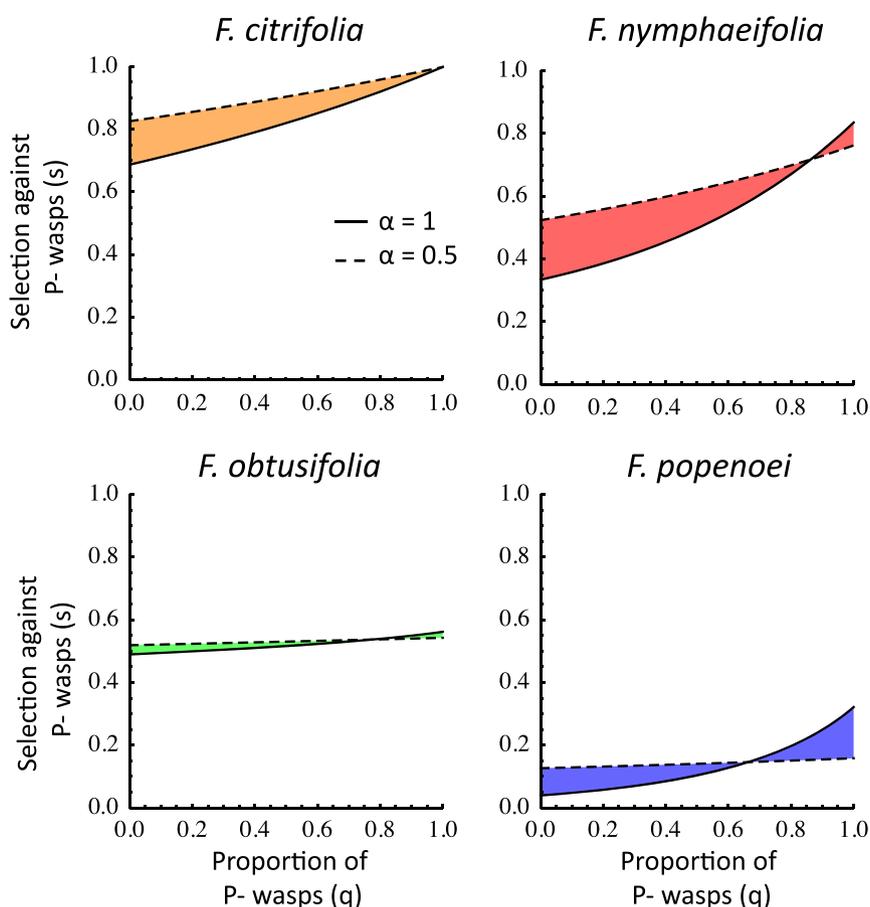


Figure 1 The strength of selection against P– wasps (selection coefficient, s) based only on costs, in four Panamanian fig species as a function of the relative frequency of P– wasps (q) and the half-saturation constant (α from 0.5 (linear) to 1 (saturating)). All species show negative frequency-dependent selection against P– wasps (see also Fig. S4).

The inbreeding coefficient F was estimated directly using microsatellites for four of the fig wasp species (Molbo *et al.* 2004); and indirectly using foundress numbers for the remaining two species (Hamilton 1979; Herre 1987; see Appendix). By rearranging eqn 3 we get the expected equilibrium proportion of P– wasps as a function of mutation rate (q_{mut}):

$$q_{mut} = \frac{F\sqrt{6s\mu} + 3\mu(1 - F)}{2s} \quad (4)$$

Results

The mutation rates required to explain the observed proportion of P– wasps (eqn 3) of each species are low and, for most of the species, within a range (5×10^{-6} to 1×10^{-4}) that is thought of as common mutation rates per loci per generation for animals (Vogel & Motulsky 1997; Drake *et al.* 1998; Nachmann & Crowell 2000; Haag-Liautard *et al.* 2007) (Fig. S5). However, the mutation rates required to explain the proportion of P– wasps in the two wasp species associated with *F. popenoei* are an order of magnitude higher than those required for the other species (Fig. S5). The value of α does not markedly affect the required mutation rates (higher α requiring higher mutation rates), except for the species with the highest foundress number (*F. popenoei*; Fig. S5).

To determine whether the proportion of P– wasps that we observe in the field (q_{field}) is due to mutations alone in these six wasp species, we calculated mutation-based estimates of q (q_{mut} , eqn 4). For these calculations, we used empirically derived values of $s(q = 0)$ and F (the mean value of F when two values were available for a wasp species) for the set mutation rates of 10^{-6} , 10^{-5} and 10^{-4} (a reasonable upper range of plausible mutation rates, erring on too high). We then compared the estimated q_{mut} with the field-collected q_{field} for each wasp species (Jandér & Herre 2010) (Fig. 2; details in Appendix).

Mutation rates of 10^{-6} and 10^{-5} give estimates of q_{mut} that are low but within the 95% CI for wasps associated with *F.*

citrifolia and *F. nymphaeifolia*, but too low for *F. obtusifolia* and much too low for *F. popenoei* (Fig. 2). In contrast, q_{mut} estimates based on $\mu = 10^{-4}$ are too high for all wasp species except those associated with *F. popenoei*, for which they are still much too low. Mutations therefore seem to be a plausible cause of q_{field} for wasps associated with *F. citrifolia*, *F. nymphaeifolia* and *F. obtusifolia*. By contrast, mutations alone cannot explain the relatively high prevalence of P– wasps observed in the field in *F. popenoei*. If we assume that all six of these closely related wasp species have a common mutation rate, we might estimate that value from the average μ of wasp species associated with *F. citrifolia*, *F. nymphaeifolia* and *F. obtusifolia*, which is 2.84×10^{-5} . The q_{mut} estimates based on this average mutation rate fall within the 95%CI of q_{field} for all wasp species except those associated with *F. popenoei* (much too low; Fig. 2). To explain the q_{field} of wasps associated with *F. popenoei*, μ would have to be 10–25 times higher than this estimated average mutation rate. Alternatively, if we relax the assumption that only a single gene is responsible, the number of genes involved to explain q_{field} in *F. popenoei* would have to be 4–8 times higher than in the other wasp species (Fig. S6, Appendix).

COST–BENEFIT BALANCE MODEL

Another possibility is that some fitness benefit of being a P– wasp balances the fitness costs imposed by host sanctions. Here, we use a game-theoretic model, in which the fitness benefit of being a P– wasp is the only mechanism counteracting the observed fitness costs, to examine how large the benefit would need to be to explain the observed proportion of P– wasps in natural populations. We assume that the system has reached an evolutionarily stable proportion of P– wasps.

To model the benefits, we consider possible fitness advantages wasps that do not pollinate (P–) could have over pollinating (P+) wasps. To pollinate a receptive fig, a P+ wasp must successfully: (1) collect pollen from her natal fig and place it in her pollen pockets, (2) carry the pollen in flight from her natal

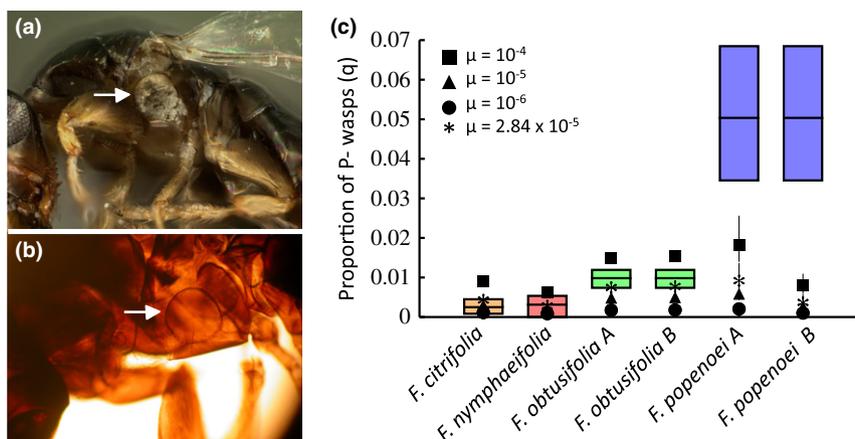


Figure 2 (a) Actively pollinating fig wasps carry pollen in thoracic pollen pockets (arrow). (b) Some wasp individuals of the pollinating species do not carry pollen (P–); their pockets are empty (arrow). (c) Comparison of the proportion pollen-free wasps in natural populations, as observed in the field (in colour, with 95% CI), and as estimated by a mutation–selection balance model with different mutation rates (μ , in black). The points for the model estimates are centered at $\alpha = 0.75$, and have error bars extending upwards to $\alpha = 1$, and downwards to $\alpha = 0.5$ (bars are only visible in *Ficus popenoei*).

tree to the receptive tree, (3) carry the pollen while moving inside the receptive fig and (4) actively distribute pollen on the stigmas using her front legs. At each of these steps, there are possible fitness advantages for P– wasps (Fig. S7, Appendix). We split the potential benefits into two types: E, those that lead to a higher likelihood for P– wasps to disperse to and encounter a receptive fig ($k > 1$), and O, those that increase P– oviposition in the fig ($w > 1$; see Appendix for details). Type E benefits include: reduced damage by males in the natal fig, reduced ant predation and increased dispersal range due to saved energy during the flight (Murray 1987; Ware & Compton 1994; Schatz & Hossaert-McKey 2003; Cook *et al.* 2015; Jandér 2015; Jandér *et al.* 2016; see Appendix). Type O benefits include: increased oviposition rate due to saved energy during the flight and due to omitting active pollen deposition (Jandér 2003; see Appendix). When including benefits in the model we evaluate each type of benefit, E and O, separately, to find out how large it would need to be to counteract the costs of being a P– wasp if that particular benefit was the only one operating. We will later discuss scenarios where multiple benefits act simultaneously.

Results

Even with no sanctions against mixed figs ($\alpha \approx 1$), the P– wasps of *F. citrifolia*, *F. nymphaeifolia* and *F. obtusifolia* must have greatly increased probabilities to encounter a receptive fig (49–217%; type E) to be able to explain their current frequencies. In all cases, these advantages must be even greater if these trees sanction mixed figs that contain both P– and P+ wasps ($\alpha = 0.75$: 73–305%; $\alpha = 0.5$: 107–474%; Fig. 3). By contrast, the relative advantages required of the pollen-free wasps of *F. popenoei* are a relatively modest 4.3% at $\alpha \approx 1$ ($\alpha = 0.75$: 8.8%; $\alpha = 0.5$: 14.6%; Fig. 3).

If we consider the advantages a pollen-free wasp must reap in terms of oviposition rate (type O), the four fig species can be divided into two categories. For the wasps of *F. citrifolia* and *F. obtusifolia*, P– wasps are not theoretically able to compensate for the fitness costs of host sanctions regardless of their oviposition rate. This is true for three reasons: (1) these species have strong sanctions against figs colonised entirely by P– wasps, (2) a high proportion of P– wasps end up in single-foundress figs, which expose them to these sanctions, and (3) a finite carrying capacity (K) limits the ability of P– wasps to compensate for sanctions by increasing their fitness in mixed figs (see Appendix section 6.5). In contrast, in *F. nymphaeifolia* and *F. popenoei* pollen-free wasps can theoretically compensate for the cost of sanctions with an increased oviposition rate. However, the required increase is very high in the wasps of *F. nymphaeifolia*: 97% greater oviposition rate at $\alpha \approx 1$ ($\alpha = 0.75$: 170%; $\alpha = 0.5$: 383%; Fig. 3). By contrast, the increases in oviposition rate required to cancel the negative effects of sanctions in *F. popenoei* are a modest 5.2% at $\alpha \approx 1$ ($\alpha = 0.75$: 10.7%; $\alpha = 0.5$: 17.9%; Fig. 3).

Incorporating drift into the cost–benefit model

In addition to the deterministic cost–benefit models, we derived a Wright–Fisher model for allele frequency change

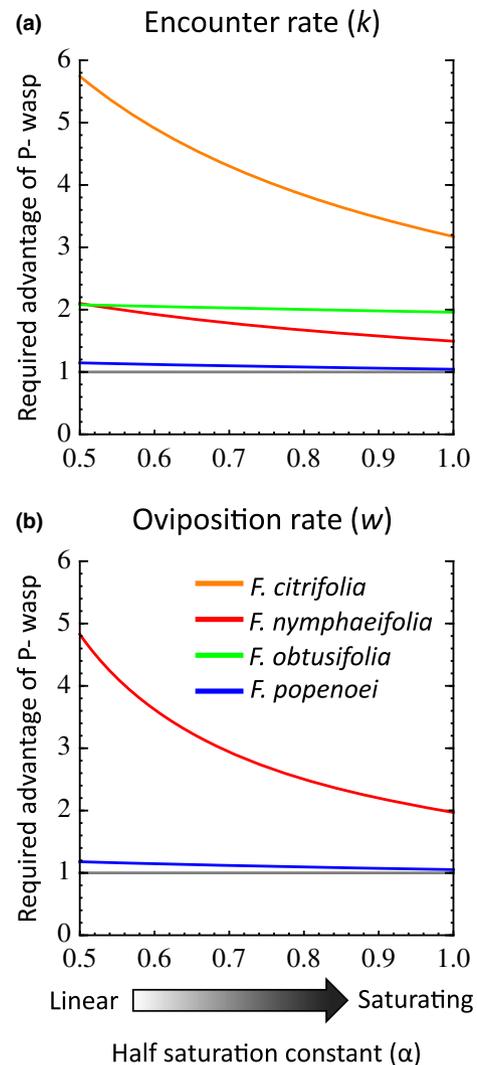


Figure 3 The required fitness benefits of P– wasps in a cost–benefit model to account for the observed proportions of P– wasps in terms of (a) the probability of encountering a receptive fig (k) and (b) oviposition rate (w). Both required advantages are expressed as a function of the half-saturation constant (α). A value of 1 indicates no relative fitness benefits are required, whereas 2 indicates that P– wasps must have double the rates of P+ wasps. For oviposition rate, the wasps of *Ficus citrifolia* and *F. obtusifolia* cannot reach the observed proportions of P– wasps irrespective of how great their advantages are.

and its diffusion approximation that incorporate the effects of genetic drift (full details in Appendix). Genetic drift can potentially impact even large populations under balancing selection when the equilibrium frequency of a rare allele is $< 20\%$ (Robertson 1962; Connallon & Clark 2012), which is the case for all our six wasp species. For each species we derived estimates of the effective population size and ran 100 iterations of the Wright–Fisher model for 100 000 generations. Each wasp species was given the required encounter rate (k , Fig. 3) to bring it to its equilibrium value in the deterministic model. To prevent these simulations from having an absorbing state (where $q = 0$), we assumed that there was always at least one copy of the ‘a’ allele in the effective population.

Simulations reveal that the P– wasp populations of *F. citrifolia*, *F. nymphaeifolia* and both cryptic species of *F. obtusifolia* repeatedly crash from the deterministic equilibrium until only a single ‘a’ allele is present (Fig. 4). By contrast, for both cryptic wasp species of *F. popenoei*, balancing selection brings P– wasp populations to near their deterministic equilibrium and maintains them there without crashes (Fig. 4). In summary, these results suggest that cheater advantages are unlikely to sustain the polymorphism in *F. citrifolia*, *F. nymphaeifolia* and *F. obtusifolia*. Even if the P– wasps reaped the very large required advantages (Fig. 3), mutations are needed to revive the populations from repeated collapse. Similarly, these simulation results provide another reason why cheater advantages are reasonable for the wasps of *F. popenoei* – once these P– wasps reach equilibrium, they stay near equilibrium even without recurrent mutation.

DISCUSSION

The mechanisms that maintain variation in symbiont quality in the fig tree–fig wasp mutualism likely differ among host species. Mutation–selection balance can maintain the low proportions of uncooperative P– wasps associated with fig species with strong and likely sanctions (*F. citrifolia*, *F. nymphaeifolia*, *F. obtusifolia*; Jandér & Herre 2010). By contrast, mutation–selection balance seems unlikely to explain the higher proportion of P– wasps associated with *F. popenoei*. However, because sanctions are weaker and less likely (due to high foundress numbers) in *F. popenoei* (Jandér & Herre 2010; Jandér *et al.* 2012), P– wasps would require only modest and

realistically sized advantages (by omitting cooperation) over P+ wasps in order to reach observed frequencies. We conclude that fitness benefits of cheating contribute to the maintenance of variability in symbiont quality only when they can be larger than the fitness costs imposed by host sanctions.

Our finding that mutation–selection balance can maintain uncooperative symbionts is consistent with previous theoretical work (Foster & Kokko 2006). The mutation rates required to account for the frequencies of P– wasps associated with the strongly sanctioning *F. citrifolia*, *F. nymphaeifolia* and *F. obtusifolia* all fall within a range (5.7×10^{-6} to 4.3×10^{-5} ; $\alpha \approx 1$) that is thought of as common mutation rates per loci per generation for animals (Vogel & Motulsky 1997; Drake *et al.* 1998; Nachmann & Crowell 2000; Haag-Liautard *et al.* 2007). In contrast, the predicted mutation rates needed by wasps associated with *F. popenoei* (3.2×10^{-4} to 7.5×10^{-4}) are an order of magnitude higher. In these calculations we assumed that a single locus determines pollination. If we relax that assumption, even lower mutation rates than those calculated here could explain the field-recorded frequencies of P– wasps (see Appendix). While we do not know whether pollination behaviour is determined by a single or multiple loci, it seems reasonable that a similar number of loci is responsible in all these closely related wasp species. The large differences in required mutation rates between *F. popenoei* and the other wasp species remain even in a multiple-loci scenario, suggesting that the pollen-free phenotype in *F. popenoei* is maintained by something other than a mutation–selection balance.

Polymorphisms maintained by mutation–selection balance typically have a very low relative frequency of the rare morph,

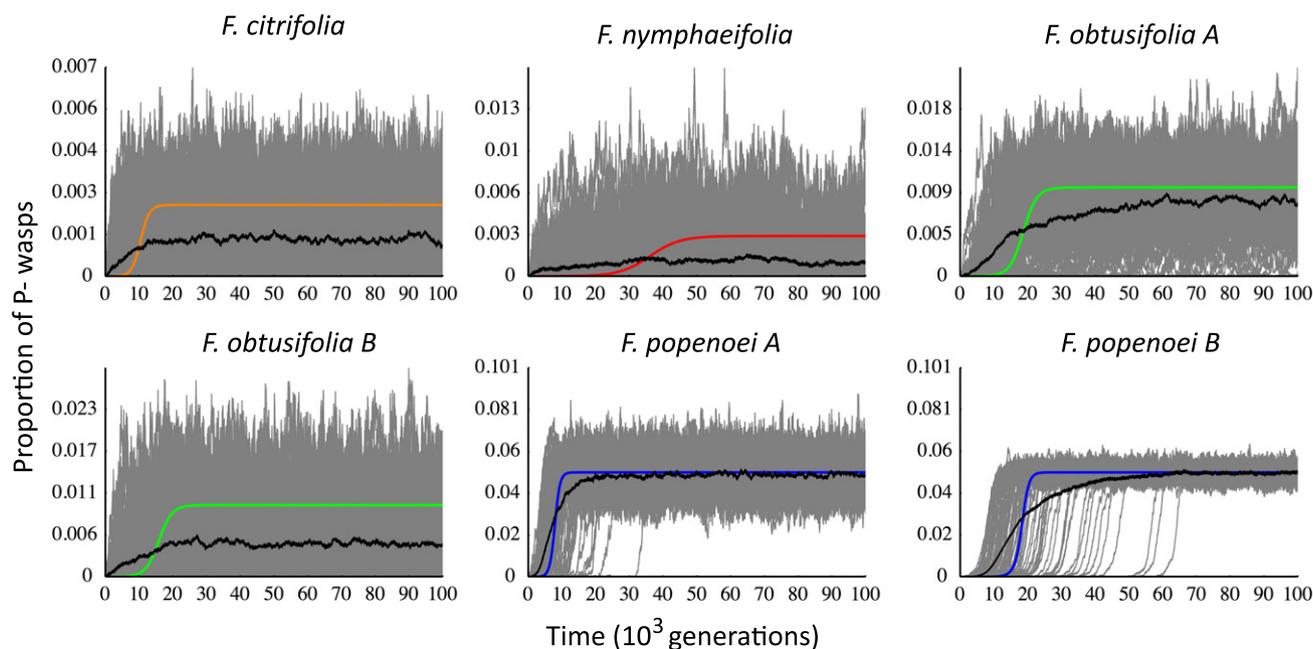


Figure 4 One hundred simulations of the Wright–Fisher model for allele frequency change for 100 000 generations (in grey) for each fig wasp species under balancing selection around q_{field} at its respective effective population size estimate [$N_e = 484\,861$ (*Ficus citrifolia*); 189 691 (*F. nymphaeifolia*); 618 294 (*F. obtusifolia A*); 210 518 (*F. obtusifolia B*); 120 119 (*F. popenoei A*); 981 886 (*F. popenoei B*)]. The deterministic model for each species is shown as a coloured line that rises from a frequency equal to a single ‘a’ allele to the equilibrium value. The thick black line gives the mean of the 100 populations in each generation. Note that populations of *F. popenoei A* (rare) and B (common) both find and stay near to the deterministic equilibrium.

e.g. haemophilia (0.02% of male births), and Tay-Sachs disease (0.03% of births in Ashkenazi Jews) (Haldane 1935; Kaback *et al.* 1993; Soucie *et al.* 1998). Similarly, in the plant *Delphinium*, albino-flowering morphs were found at 0.07% (Waser & Price 1981). The much higher frequencies of pollen-free fig wasps in this study (0.3–1%, Jandér & Herre 2010) are still realistically explained by mutation–selection balance because two factors, inbreeding and selection acting only on females, combine to increase their prevalence.

In hosts with weak sanctions the situation is different. Theoretical models demonstrate that cheater symbionts can coexist with mutualists when discrimination is imprecise (Friesen & Mathias 2010; Steidinger & Bever 2016), costly (Foster & Kokko 2006; Steidinger & Bever 2014) or relaxed when mutualists generate surplus services (Bever 2015). These models require that cheater symbionts have higher fitness than mutualists in the absence of host discrimination. However, while uncooperative symbionts have been shown to enjoy higher growth rates (Bennett & Bever 2009; Bever *et al.* 2009; Sachs *et al.* 2010) and survivability (Ratcliff *et al.* 2008) than cooperative ones in some plant–microbial mutualisms, it remains unclear if such cheater advantages are sufficient to explain the frequency of uncooperative symbionts in the field.

Our study is the first to integrate empirical data with theoretical models to determine the required advantages cheater symbionts must possess in order to coexist with mutualists. We conclude that in strongly sanctioning fig species, P–

wasps either cannot compensate for the costs of sanctions by increasing their oviposition rates (*F. citrifolia* and *F. obtusifolia*), or else require unrealistically large advantages over P+ wasps in order to reach observed frequencies (e.g. 100% faster oviposition or 50 to > 200% more likely to encounter a receptive fig). By contrast, the observed frequencies of P– wasps associated with the weakly sanctioning *F. popenoei* are likely to be maintained by trade-offs between the benefits of avoiding costly symbiotic services (pollination), and the sanction-induced fitness costs of not pollinating. When the presence of a single P+ wasp is sufficient to avoid sanctions ($\alpha = 1$), P– wasps in *F. popenoei* must be only 4.3% more likely to encounter a receptive fig or have 5.2% higher oviposition rates in order to occur at the observed frequencies. These benefits are in credible ranges. For example, behavioural observations suggest that by omitting the pollination movements wasps would save 2–5% of time inside a fig, thereby being able to lay more eggs (these wasps are often limited by time rather than eggs; Jandér 2003; Jandér unpublished). Combinations of benefits are entirely possible, both within benefit types and across them. For example, a P– wasp might both benefit from avoiding ant predation and by carrying less weight in flight, both of which may increase the likelihood of reaching a flowering fig (k). The estimated k is then made up of both types of benefits (at undetermined proportions), requiring a lower contribution from each individual benefit than if benefits were acting singly. The field-collected pollen-free wasps

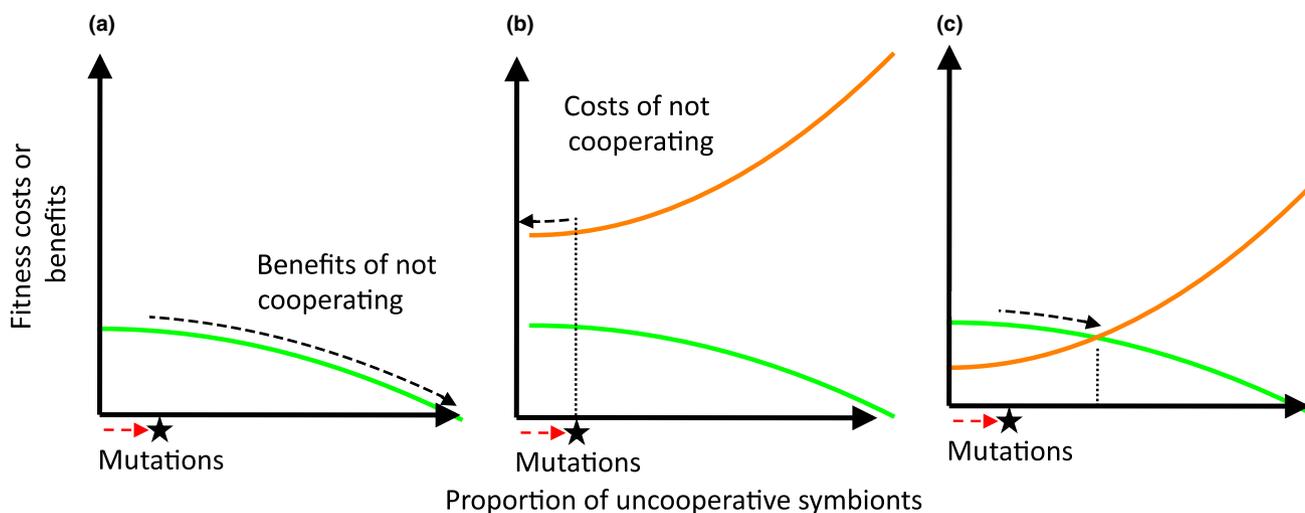


Figure 5 Suggested general explanation for the proportion of uncooperative symbionts (q) found in a mutualistic system. (a) Mutations (red arrow) cause a proportion of symbionts (star) to be ineffective/uncooperative. If there are fitness benefits associated with not cooperating (e.g. rhizobia saving energy due to not fixing nitrogen, or fig wasps not carrying pollen in flight), the proportion of uncooperative symbionts would increase in the population (dashed arrow). The fitness benefits of not cooperating (green line) may be unaffected by q , or may be declining as q increases if the relative advantage uncooperatives have over other symbionts decreases as q increases. (b) If there are fitness costs of not cooperating (orange line), those costs will push q back towards 0 (dashed arrow), but new mutations in each generation will keep adding uncooperative symbionts. In situations where fitness costs of being uncooperative are higher than the benefits, q will therefore be determined by a mutation–selection balance, determined by the costs of not cooperating, and the mutation rates. Costs are likely to increase with q because as uncooperative symbionts increase in frequency they can less often free-ride on neighbouring cooperating symbionts (Friesen & Mathias 2010; Jandér *et al.* 2012). Fitness costs of not cooperating do not have to be due to host sanctions/selective resource allocation; they could also be due to an absence of automatic fitness feedbacks from the host, or be due to uncooperative symbionts being generally defective. (c) If fitness benefits of being uncooperative are higher than the costs (e.g. due to weak or ineffective sanctions, or very costly cooperation), q would increase until the costs of not cooperating balance the benefits (lines cross). Under this scenario, mutation rates provide the initial variability in symbiont quality, but they do not determine the equilibrium level of q .

documented in Jandér & Herre (2010) must be either $P-\alpha$ or $P-\beta$ (Fig. S7), so they must minimally gain any benefits from not carrying pollen in flight, and may enjoy other benefits in addition.

Increasing the extent to which mixed figs (with both $P+$ and $P-$ wasps) are sanctioned ($\alpha < 1$) makes host sanctions more effective. In the mutation–selection and cost–benefit models, this means higher required mutation rates and cheater advantages, respectively. This is consistent with findings from game-theoretical models of linear vs. nonlinear public goods (Archetti *et al.* 2011) as well as module-level partner choice in mutualisms (Steidinger & Bever 2016). However, the true α for the species investigated here is likely 1 or close to 1; even in the strongly sanctioning *F. nymphaeifolia*, having merely one pollinator in each fig: (1) ceases abortions, (2) brings the number of offspring up to almost the same level as figs with only pollinators and (3) bring wasp size to the same level as in figs with only pollinators (Jandér *et al.* 2012; Jandér & Herre 2016).

Variation in pollination efficiency could be caused by both variation in the environment (such as pollen scarcity) and variation in wasp behaviour. Although the pollen-free wasps studied here likely are caused by a heritable trait rather than environmental variation, there are other examples of wasps that vary in their pollination efficiency for environmental reasons. In the Asian actively pollinated *F. montana*, seed set was low when pollen was scarce in the foundress's natal fig (Kjellberg *et al.* 2014). Wasps that fail to collect sufficient pollen for any reason (mutations or environment) are likely to help maintain host sanctions, and thus help stabilise the mutualism (Foster & Kokko 2006). Because host sanctions likely are based on selective resource allocation to fruits that are better pollinated and therefore more beneficial for the tree (Jandér & Herre 2016), they are likely less costly to maintain than are partner choice mechanisms that have evolved purely in response to uncooperative symbionts (Foster & Kokko 2006). Nevertheless, sanctions are likely to incur some costs; for example, sanctions are not present in passively pollinated fig species where failing to carry pollen is practically impossible (Jandér & Herre 2010). In this study we do not attempt to establish why fig species differ in sanction strength, only the effect these sanctions have on wasp fitness.

Although the data in this study were obtained from a few species of figs and their pollinating wasps, the general conclusions are applicable both to other actively pollinated fig species, and to other mutualisms with host sanctions and costly services such as the legume–rhizobia mutualism and plant–mycorrhizal mutualism (Kiers *et al.* 2003, 2011; Simms *et al.* 2006; Bever *et al.* 2009; Werner & Kiers 2014; Bever 2015). The models presented here could be modified to fit other mutualistic systems, and they motivate future empirical investigations of the prevalence of uncooperative symbionts, the frequency of mixed infections, the strength and precision of host sanctions and the benefits of not cooperating. We propose that the frequencies of uncooperative symbionts are likely governed by mutation rates in species where hosts can impose sufficiently strong or directed sanctions, making the costs of not cooperating larger than the benefits (Fig. 5b). On

the other hand, in hosts where sanctions are weak or imprecise, uncooperative symbionts may proliferate until they reach the equilibrium frequency at which costs and benefits of not being cooperative are balanced (Fig. 5c). Because both cases are illustrated here within a single type of mutualism, we suggest that the mechanisms that resolve the paradox of uncooperative symbionts likely differ among host species.

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AUTHORSHIP

KCJ designed the study, collected data and wrote the first draft. Both authors constructed the models, analysed the data and edited the paper.

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