



Tansley review

Conflict, cheats and the persistence of symbioses

Author for correspondence:

Angela E. Douglas

Tel: +44 1904 328610

Fax: +44 1904 328505

Email: aed2@york.ac.uk

Received: 29 August 2007

Accepted: 30 October 2007

Angela E. Douglas

Department of Biology, University of York, York, YO10 5YW, UK

Contents

| | | | |
|--|-----|-----------------------------------|-----|
| Summary | 849 | IV. Routes to conflict resolution | 854 |
| I. Introduction | 850 | V. Discussion | 856 |
| II. Conflict and cheats | 850 | Acknowledgements | 857 |
| III. Symbiotic interactions without cost | 852 | References | 857 |

Summary

Key words: byproduct mutualism, conflict, reciprocity, sanctions, symbiosis, vertical transmission.

Many symbioses are widespread, abundant, and evolutionarily persistent. This is despite unambiguous evidence for conflict between the partners and the existence of cheats that use benefits derived from their partners while providing reduced or no services in return. Evidence from a diversity of associations suggests that symbioses are robust to cheating in several ways. Some symbioses persist despite conflict and cheating because of the selective advantage of cost-free interactions (also known as byproduct mutualistic interactions), which incur no conflict. There is also evidence for the suppression of cheating by sanctions imposed by partners in some symbioses, and vertical transmission has been shown experimentally to promote traits that enhance partner performance. It is argued that these processes contribute to the apparent rarity of evolutionary transitions from symbiosis to parasitism. There is strong phylogenetic evidence for the evolutionary reversion of various symbiotic organisms to free-living lifestyles, but at least some of these transitions can be attributed to selection pressures other than within-symbiosis conflict. The principal conclusion is that, although conflict is common in symbioses, it is generally managed and contained.

New Phytologist (2008) **177**: 849–858

© The Author (2008). Journal compilation © *New Phytologist* (2008)

doi: 10.1111/j.1469-8137.2007.02326.x

I. Introduction

Things fall apart. This is the fate of mutually beneficial relationships (mutualisms), as predicted in the traditional ecological and evolutionary literature. These predictions are not borne out by the facts. Examples of persistent symbioses include: the roots of >75% of all vascular plant species estimated to be mycorrhizal with fungi (Trappe *et al.*, 1987), including the arbuscular mycorrhizal fungal symbiosis which evolved *c.* 400 million yr ago (Simon *et al.*, 1993; Redecker *et al.*, 2002); the coral–algal symbiosis which probably evolved *c.* 240 million yr ago and today underpins essentially all wave-resistant, calcareous reefs in shallow, clear waters at low latitudes (Woods, 1999); and all modern eukaryotes, which have descended from a 1–2 billion-yr-old symbiosis between eukaryotic cells and the ancestor of mitochondria (Embley & Martin, 2006).

The interesting aspect of the traditional but erroneous perspective is the explanations given for the predicted rarity and evolutionary transience of symbioses. The ecologists argued from the Lotka–Volterra equations that mutualisms are ecologically unstable because an increase in the number of one organism in a relationship would promote an increase in the population of its partner. Antagonistic interactions, such as competition, are expected to generate co-existing populations that are stable or fluctuate in predictable ways. The evolutionary argument for the instability of symbioses is different: genotypes that confer benefit on nonkin are at a selective disadvantage relative to ‘selfish’ genotypes that provide no benefit. In other words, symbioses were argued to fail in ecological time because they are too mutualistic and in evolutionary time because they are not mutualistic enough. Each argument makes the other implausible, and both arguments can be rebuffed by recognizing that, although among-partner conflict is a common feature of symbioses, it is generally managed and contained.

Until relatively recently, rather little attention was paid to conflicts in symbioses. The reason is that symbioses have tended to be considered as mutually beneficial associations and alternatives to antagonistic relationships, such as parasitism. This perspective has discouraged research on similarities and parallels between symbioses and overtly parasitic relationships. Current interest in conflict and antagonism in symbioses is linked with an increasing acceptance of the original definition of symbiosis as any persistent association between different species, and arises from the recognition that many real relationships are not invariably either mutualistic or antagonistic. The impact of an association on an organism can vary with environmental conditions and with the genotype, age and condition of the partners, and this variation can include organisms with effects that vary from beneficial to neutral and deleterious. For example, the fungus *Colletotrichum magna* was first isolated as a virulent plant pathogen, but is now known to promote

growth, drought tolerance and resistance to fungal pathogens for some plant species (Redman *et al.*, 2001). Even the arbuscular mycorrhizal fungi, which are usually regarded as highly specialized beneficial symbionts of plant roots (see above), can occasionally be harmful to the plants (e.g. *Glomus macrocarpum* causes a stunting disease of tobacco plants; Modjo & Hendrix, 1986). Furthermore, a single mutation can transform a symbiont that promotes the growth and vigour of its partner into a pathogen, or vice versa. Inactivation of the gene *noxA* coding NADPH oxidase of the endophytic fungus in perennial ryegrass *Lolium perenne* caused dramatic increase in fungal biomass and stunted growth and premature senescence of the grass (Tanaka *et al.*, 2006a). (The underlying mechanism is uncertain but reactive oxygen species generated by NADPH oxidase may activate fungal signalling pathways that regulate cell proliferation.)

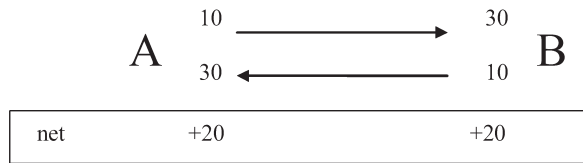
The purpose of this article is to examine the incidence and consequences of conflict in symbioses. It does not seek to be comprehensive, and readers are referred to several excellent reviews for complementary perspectives (Ferriere *et al.*, 2002; Sachs *et al.*, 2004; West *et al.*, 2007). The sources of conflict and cheating are addressed in section II, and the significance of symbiotic interactions that are not liable to conflicts is explored in section III. Section IV considers the routes by which conflict is resolved and cheats are suppressed. The incidence of symbiosis breakdown offers one way to assess the effectiveness of conflict management in symbioses, and this is one of the topics explored in the Discussion (Section V).

II. Conflict and cheats

Most research on the conflict between partners in a symbiosis is focused on the costs of providing a service to the partner. As illustrated in Fig. 1a, this cost is deemed acceptable to the organism when it is smaller than the benefit obtained from the reciprocated service provided by the partner. The interactions in Fig. 1a are variously described as reciprocity, reciprocal benefit and reciprocal altruism. (The term reciprocal altruism is potentially misleading because there is nothing altruistic in this type of interaction.) Symbioses are widely assumed to be founded on such reciprocal exchange.

There is, however, an obvious difficulty with reciprocity, namely that any organism gains more by failing to reciprocate, as is illustrated by Fig. 1b. Organism A is a ‘cheat’ because it gains more by being ineffective as a partner in a symbiosis. The conflict encapsulated in Fig. 1b is logically identical to the Prisoner’s Dilemma game (Axelrod, 1984). As research on the Prisoner’s Dilemma game has revealed, this conflict between the individual advantage of cheating and collective benefit of providing a service can be resolved in favour of reciprocity by repeated interactions (Axelrod, 1984). In particular, a partner that is ‘nice’ (starts by cooperating) but ‘provokable’ (punishes a partner that cheats) promotes

(a) The cost of reciprocal benefit



(b) The 'temptation' to cheat

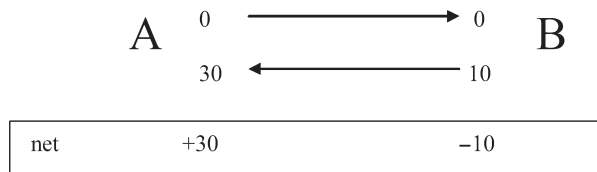


Fig. 1 The cost of reciprocal benefit between two organisms (A and B). (a) The cost of reciprocal benefit: each organism provides a service at cost of 10 arbitrary units, which has a value of 30 units to the recipient, yielding a net benefit of 20 units for each organism. (b) The 'temptation' to cheat: organism A does not provide the service and obtains increased net benefit (30 units) to the detriment of organism B, which derives a net cost of 10 units.

cooperation in partners and performs well by rarely losing. The realism of these models for symbiosis can be increased further by allowing partners to 'opt out' of interactions with partners that cheat. These models suggest that repeated or persistent interactions are likely to dampen conflict in reciprocal interactions.

The many models exploring conflict in symbioses presuppose that it is costly for organisms to provide a service to their partner. This assumption is undoubtedly valid for many nutritional interactions involving symbiotic microorganisms. For example, the dinoflagellate alga *Symbiodinium* releases an estimated 90% of its photosynthetic carbon (C) to its coral hosts (Muscatine *et al.*, 1984); and up to 50% of the essential amino acids synthesized by the bacterium *Buchnera* is transferred to its aphid hosts (Douglas *et al.*, 2001). The costs can also be substantial for symbioses between animals and plants, notably for plants pollinated by insects, which are also seed predators. For example, figs (trees of the genus *Ficus*) are pollinated exclusively by agaonid wasps, which lay eggs in some of the flower ovules, thereby diverting them from seed production to wasp production. The details of *Ficus*–wasp interactions vary widely, with some *Ficus* species providing specialized 'neuter' flowers for wasp oviposition and some wasp species actively harvesting pollen into specialized pollen pouches, but the core cost remains: *Ficus* reproduction is dependent on pollinators that consume seeds (Pellmyr, 2002). To the first approximation, the cost in this symbiosis is the number of predated seeds.

Conflict generates the selective opportunity for cheats, organisms that reduce their costs through conferring little or no benefit on their partner, and thereby enhance their own

fitness. Interactions involving readily observable behaviour of animals, especially cleaner and pollination systems, have provided some of the best demonstrated instances of cheating.

In every encounter with a client, a cleaner has a choice whether to forage for ectoparasites or to cheat by consuming healthy skin and flesh of its client. The cost of a cheating cleaner to the client is persistent ectoparasites, often compounded by direct damage, while the advantages to the cleaner are reduced foraging time and possibly higher quality food. Oxpecker birds (*Buphagus* spp.) remove ectoparasites, especially ticks, from gazelle and other ungulates, but they also peck at damaged skin and scabs on some clients to keep skin wounds open, and then feed on the blood (Mooring & Mundy, 1996). The cleaner fish *Laboroides dimidiatus* regularly cheats on its clients by 'taking a bite', although the incidence of cheating varies among individual cleaners and client species (Bshary, 2002) and, remarkably, is reduced in cleaners that can see other potential clients 'watching them' (Bshary & Grutter, 2006).

The 'temptation' for a pollinator to cheat comes from the high foraging costs imposed by flowers in which the nectaries are relatively inaccessible, for example at the far end of a corolla (a floral tube made up of fused petals). This arrangement is to the plant's advantage because it enhances the probability of contact between the pollinator and flower's reproductive structures but the foraging animal can reduce the time required to obtain nectar by boring or piercing a hole through the corolla at the site of the nectaries without contacting the flower's reproductive structures. Opportunistic nectar robbers include bees and hummingbirds, which are often followed by secondary robbers that cannot make the holes but exploit pre-existing damage to flowers (Pellmyr, 2002). Honeybees are very adept secondary robbers, and this is one factor contributing to the inefficiency of honeybees as pollinators (Westerkamp & Gottsberger, 2000) (the honeybee has been selected for honey production, not pollination). Studies of *Bombus* bumblebees indicate that some individuals may alternate between feeding 'legitimately' via the flower mouth and illegitimately through the damaged corolla, while other individuals may be either specialist pollinators or specialist robbers; the reasons for these different strategies are not understood fully (Maloof & Inouye, 2000). However, we should be cautious in assuming that all nectar robbers are detrimental to the plant. Some robbers do not reduce seed set of plants (Maloof & Inouye, 2000) and a 5-yr study of the vetch *Anthyllus* in Spain revealed that nectar-robbing *Bombus* actually promote pollination and seed set because they are 'heavy-footed', effecting pollen transfer as they clumsily rob the nectaries (Navarro, 2000).

Not all cheating is opportunistic. Many symbioses are exploited by species that invariably exploit the association without conferring any benefit to the partners. Some of these 'professional' cheats are closely related to a partner in a symbiosis, and may have evolved from the symbiotic partner of the taxon with which they are associated. Bronstein (2001)

calls this 'defection from mutualism'. Others are distantly related and are presumed to have had no evolutionary history of involvement in the symbiosis that they exploit, although they could be derived from a symbiotic partner that has subsequently become extinct. Following Bronstein (2001), these can be called 'third-lineage' cheats.

Defection from mutualism is illustrated by the seed predator pollination of *Yucca* trees by *Tegeticula* moths, a relationship broadly similar to the association between figs and fig wasps considered earlier. The moths pollinate the plant actively. An adult female collects pollen from one flower and then flies to another flower at a different developmental stage, where she forces the pollen down the receptive stigma with her mouthparts and then deposits eggs via the ovipositor. Detailed morphological and molecular studies have revealed that *Tegeticula* comprises at least 16 species, two of which have secondarily abandoned pollination and so made the transition from mutualist to cheat (Pellmyr *et al.*, 1996; Pellmyr & Leebens-Mack, 2000; Althoff *et al.*, 2006). The persistence of these two species depends on the mutualists, which ensure that the host plant reproduces. Even so, the cheats can substantially depress seed set of the *Yucca* (in one study by up to one-third; Pellmyr & Leebens-Mack, 2000).

The fig–fig wasp relationship is also subject to nonpollinating cheats, predominantly third-lineage cheats (Cook & Rasplus, 2003). Indeed, a single fig species pollinated by just one or two species of agaonid wasp species can be exploited by up to 30 nonpollinating wasp seed predators that are not closely related to agaonids (although all are chalcidoid wasps, the superfamily that also includes the agaonids). There is persuasive molecular evidence that the cheating habit has evolved multiple times (Marussich & Machado, 2007).

Many third-lineage cheats are highly specific. The ant–plant symbiosis between the ant *Petalomyrmex phylax* and the West African tree *Leonardoxa africana* has been invaded by a cheating ant *Cataulacus mckeyi*, which colonizes the plant's domatia (hollow portions of stem in which the ants nest). Unlike the mutualistic ant partner, which actively patrols the plant, *C. mckeyi* is relatively inactive, only travelling from its nest site to feed on the ant-bodies and extrafloral nectaries (Fig. 2; Gaume & McKey, 1999). Cheats also exploit the relationship between plants of the genus *Piper* and the ant *Pheidole bicornis*. Unusually, *Piper* plants produce ant-food bodies only when colonized by their ants. This symbiosis is invaded by beetles of the genus *Phyllobaenus*, which consume both the ants and the food bodies while providing no protection for the plant. Furthermore, it induces the plant to continue producing food bodies; even *Piper* plants with no prior experience of ants produce food bodies for the cheating beetle (Letourneau, 1990).

Among symbioses involving microorganisms, by far the best-characterized cheats are some mycoheterotrophic plants. Mycoheterotrophs are plants that derive fixed carbon (C) from fungi. Some mycoheterotrophs exploit saprophytic

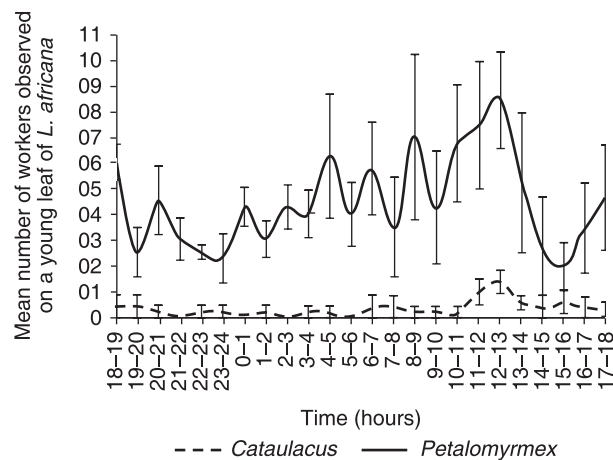


Fig. 2 The activity rhythms of ants *Petalomyrmex phylax* (mutualist) and *Cataulacus mckeyi* (cheat) in symbiosis with the plant *Leonardoxa africana*. (Reproduced from Fig. 4 of Gaume & McKey (1999) with permission from Blackwell Publishing)

fungi (the seedlings of many orchids are examples), but the plants of interest here are those that exploit mycorrhizal fungi. By tapping into an established mycorrhizal fungal network, the mycoheterotroph gains both inorganic nutrients, such as phosphate, from the fungus and C fixed by other plants associated with the fungus. Mycorrhizal symbioses are exploited in this way to provide the total C requirements of an estimated 400 species of nonphotosynthetic plants from 10 families (Leake, 2004). They include cheats of both ectomycorrhizas (e.g. some orchids and *Monotropia*) and arbuscular mycorrhizas (e.g. the South American plant *Arachnitis*; Taylor & Bruns, 1997; Bidartondo *et al.*, 2002). Furthermore, various green orchids, which live in deep shade in woodlands, also exploit mycorrhizas to meet part of their C requirements (Julou *et al.*, 2005). A common feature of the mycoheterotrophic plants which exploit mycorrhizas is the small taxonomic range of fungi with which each species associates, in contrast to the very broad range used by most fully photosynthetic plant species. This difference suggests that the mycorrhizal fungi may be under selection pressure to be resistant to these cheats.

To summarize, conflict and the linked selection for cheating occur widely across a range of different types of symbioses. However, this does not mean that conflict is a universal feature of symbiotic interactions. The next section explores some services that appear to be provided without any cost incurred by the donor.

III. Symbiotic interactions without cost

Cost-free traits are displayed by an organism entirely for itself and access by other organisms is an incidental byproduct. For this reason, cost-free services are also known as byproduct mutualism (Conner, 1995). The classic example of cost-free

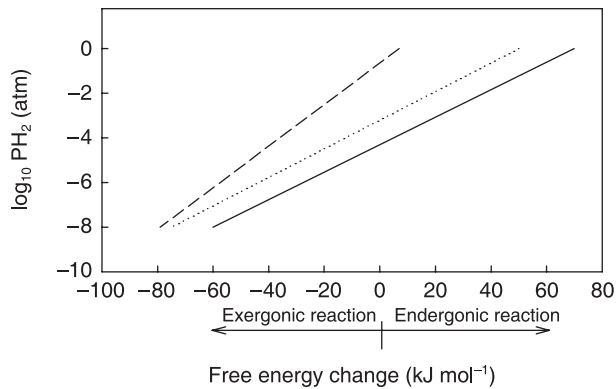


Fig. 3 The metabolic basis of hydrogen transfer between species provides an example of byproduct mutualism based on the impact of partial pressure of hydrogen on the free energy change associated with the oxidation of three different organic substrates, propionate (solid line), butyrate (dotted line) and ethanol (dashed line). (Redrawn from Fig. 2.24 of Fenchel & Finlay (1995) with permission from Oxford University Press.)

cooperation is the 'selfish herd' (herd, flock, school, etc.). By joining a group, each individual reduces the risk of predation for itself and all the other members of the group. There is no conflict among members of the 'selfish herd'.

Most discussion of byproduct mutualism has focused on behavioural traits of animals and the reviews of Conner (1995) and Sachs *et al.* (2004) provide many examples. This type of interaction is also potentially significant in symbioses involving microorganisms, especially those living in anoxic environments. A metabolic problem faced by many anaerobes is that the fermentation of organic compounds such as short-chain fatty acids and alcohols requires a low partial pressure of hydrogen (pH_2); at high pH_2 , the oxidation of these compounds is energy-consuming (Fig. 3). One route to maintain a low pH_2 is to form a symbiosis with hydrogen-scavenging bacteria, such as methanogens, which use hydrogen as a substrate for ATP production. The transfer of hydrogen from the fermenting bacterium to the methanogen is beneficial to both partners: reducing the pH_2 in the first partner and providing a sustained source of hydrogen substrate for the second partner. The benefit to the partner is incidental for other organism. Mutual benefit based on hydrogen transfer has been identified in various consortia between bacteria and between anaerobic protists, especially ciliates, and intracellular methanogens (Fenchel & Finlay, 1995).

Nitrogen (N) recycling between animals and microorganisms also illustrates the difference between cost-free interactions and costly interactions. Nitrogen recycling has been invoked for animal symbioses with algae, including the dinoflagellate algae *Symbiodinium* in corals (Tanaka *et al.*, 2006b) and prasinophyte algae *Tetraselmis* in the acoel flatworm *Symsagittifera* (= *Convoluta*) *roscoffensis* (Douglas, 1981), and in insect symbioses with bacteria, including the

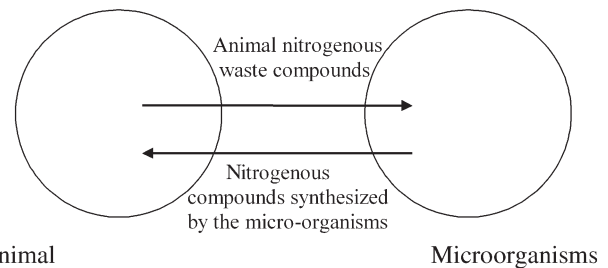


Fig. 4 Nitrogen recycling in animal-microbial symbioses.

The microorganisms (collectively displayed here as a circle) transform nitrogenous waste products of the animal (ammonia, urea, etc.) into nitrogenous compounds valuable to animal metabolism, and these compounds are translocated back to the animal tissues.

cockroach-*Blattabacterium* association (Mullins & Cochran, 1975). This interaction comprises two steps: a cost-free transfer from the animal to its symbiotic microorganisms and a costly transfer in the reverse direction (Fig. 4). Both partners benefit from microbial consumption of animal nitrogenous waste compounds. The animal benefits because the microbial symbionts act as a sink for potentially toxic nitrogenous waste compounds and the symbionts benefit from access to a N source for growth. This interaction would only become costly if the animal produced more ammonia than is dictated by its own metabolism just to feed its symbionts, or if the symbionts consumed the ammonia at rates higher than dictated by their own N requirements just to reduce the ammonia load on the animal. We have no evidence that either partner 'invests' in the interaction in this way although it appears that this possibility has not been excluded definitively for any association.

Nitrogen recycling is not a futile symbiotic cycle because the microorganisms are metabolically more versatile than their animal host. In particular, they can use the animal-derived nitrogenous compounds as substrates to synthesize nitrogenous compounds that the animal cannot produce. When the microorganisms release these compounds back to the animal they have recycled the N. Recycled compounds valuable to the host include essential amino acids (i.e. the nine amino acids which contribute to protein but cannot be synthesized by animals). This second step is costly to the symbionts because essential amino acid transfer to the host reduces the pool of these nutrients to support microbial growth.

Symbiotic interactions that incur no costs have been studied rather little, probably because they lack the evolutionary conflicts and dilemmas inherent in reciprocal exchange of costly services. Nevertheless, these cost-free interactions may make an important contribution to the persistence of symbioses with costly interactions that incur conflict and cheating. The implication is that, although conflict is probably widespread, it is not a universal feature of symbiotic interactions. In symbioses, some things really are free.

IV. Routes to conflict resolution

A core question for symbiosis research is: Why don't 'things fall apart'? As considered in Section III, the costs of conflict might be tolerated in some symbioses because of the selective importance of cost-free interactions. This section turns to other factors promoting the persistence of symbioses. There is now excellent evidence that partners in symbioses respond to conflicts in at least three different ways to reduce conflict and its negative consequences.

1. Sanctions and rewards

There is considerable current interest in the possibility that organisms in symbiosis might reward partners that provide a service and punish those that do not. This perspective presupposes that the partnership is asymmetric, with one controlling organism that enforces the 'good behaviour' of its partner(s). From first principles, the controller is expected to have the least incentive to cheat. This could be the organism with the greatest selective interest in the relationship or the one that incurs the lowest costs. For many associations involving a single, large host and many small organisms, the controller is the host. This is because the smaller partner generally has less invested in the current relationship, as a result of its higher intrinsic reproductive rate and the greater number of propagules it can produce to disperse away from the association. Differential costs may be important, for example, in mycorrhizas where the plant is likely to be the controller and may incur little cost. Although *c.* 20% of photosynthetically fixed C is allocated to the fungal partner, at least from heavily-colonized plants grown under laboratory conditions, this probably does not represent a substantial cost for the plant because photosynthesis is commonly sink-limited (Bryla & Eissenstat, 2005; Kiers & van den Heijden, 2006).

A potentially important 'control point' in some symbioses is the control by one organism over the supply of nutrients required for the growth and persistence of its partners. For all intracellular symbionts, the profile of nutrients acquired is determined by the transport properties of the bounding host membranes and metabolic traits of the host cell. Additionally, the nutritional requirements of some symbiotic organisms, including many intracellular symbionts, can only be met in the association. For example, the symbiotic bacteria in some insects have a very restricted capacity to synthesize amino acids and nucleotides (Zientz *et al.*, 2004), and the arbuscular mycorrhizal fungi have no capacity for saprophytic growth in soils and are dependent on plant-derived sugars for C (Smith & Read, 1997). This asymmetry provides a route by which the controlling partner can reward partners that provide benefits and punish cheats. For example, Fitter (2006) has proposed that the plant rewards arbuscular mycorrhizal fungi which provide phosphate by supplying them with

photosynthetic C; the more phosphate provided, the more C allocated. This reward scheme is founded on known plant physiology. Roots respond to phosphate-rich patches in the soil by increasing the allocation of photosynthate, supporting root proliferation. Mycorrhizal colonization, however, tends to inhibit the initiation of new roots. It is hypothesized that the plant allocates C to the phosphate-rich patch at the arbuscule-plant interface, and this C is consumed by the fungus. A more complex nutritional reward scheme based on amino acid metabolism has been proposed to ensure sustained release of N fixation products from rhizobia (Lodwig *et al.*, 2003).

Some control measures in symbioses exclusively comprise sanctions against cheating partners. When the *Tegeticula* moths deposit many eggs, the *Yucca* plants abort their fruits (Pellmyr, 2002), and cleaning fish that cheat on their clients are often chased away (Bshary & Grutter, 2005). One of the best-studied instances of sanctions comes from the experimental study of Kiers *et al.* (2003) on the symbiosis of the N-fixing rhizobial bacteria *Bradyrhizobium* with soybean plants. The rhizobia bacteria were prevented from fixing N by replacing air with a N-free atmosphere of argon and oxygen. The response of the symbiosis was a reduction in the numbers of rhizobia, whether the experiment was conducted at the scale of the whole root, part of the root system or even the individual root nodule (Fig. 5a). This was correlated with a reduction in the oxygen tensions in the central infected zone of the nodule where the rhizobia are located and depressed oxygen permeability of the outer nodule tissues (Fig. 5b). It appears that the legume plants can impose sanctions on rhizobia that fail to fix N and that the sanctions include decreasing the oxygen supply to the rhizobia.

2. The opportunity to cheat

The incidence of cheating depends on opportunity, and this can be minimized, as various ant-plant relationships illustrate. The ants have no short-term selective interest in the reproductive output of the plant and may even benefit from depressed plant reproduction because flower and fruit production consumes plant resources that could otherwise be allocated to vegetative growth and production of ant foods. This is presumably why the *Allomerus* ants, which protect the South American understorey plant *Cordia nodosa* from herbivores, also systematically attack and destroy flower buds, preventing fruit production (Yu & Pierce, 1998). These ant colonies exploit the increased growth of their castrated plant hosts to produce elevated numbers of domatia (ant nest-sites) and ants, including winged individuals for dispersal. This outcome of the conflict between ants and plants is not, however, the norm because many plants display various adaptations which reduce ant access to flowers. For example, *Hirtella myrmecophila* hosts *Allomerus* ants in pouches on young leaves, but these pouches are lost by abscission when the leaves

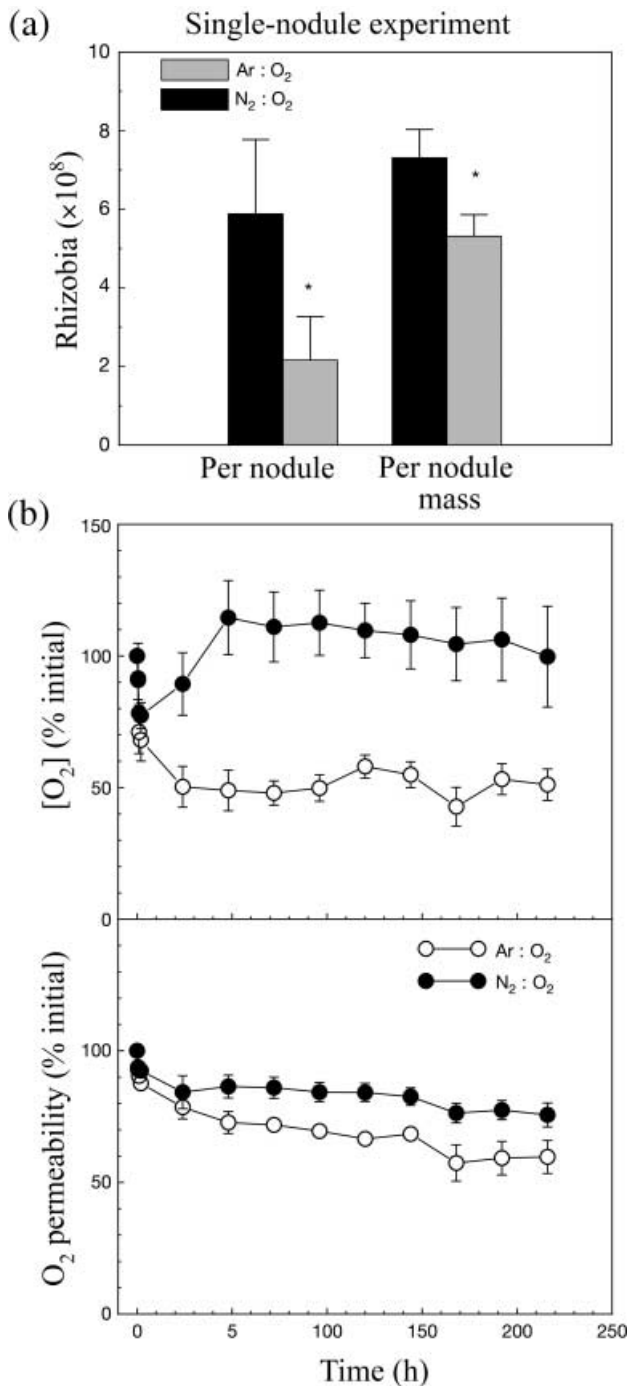


Fig. 5 Sanctions imposed by soybean plants on *Bradyrhizobium* symbionts forced to cheat by exposure to nitrogen-free air (Ar : O₂, with nodules in N₂ : O₂ air as controls). (a) Number of rhizobia in nodules; (b) oxygen relations in nodules. (Reproduced from Figs 2 and 3 of Kiers *et al.*, 2003. Reprinted with permission from Macmillan Publishers Ltd: Nature 2003.)

mature. As a result, the flowers of *H. myrmecophila*, which are borne exclusively on branches bearing old leaves, are unlikely to be contacted by the potentially castrating ants (Izzo & Vasconcelos, 2002).

3. Persuasion

Just as conflict arises from a divergence in selective interest between the partners of a symbiosis, conflicts can be resolved by increasing the overlap in selective interest of the partners. The chief route to selective overlap is vertical transmission; the fittest symbiont is the one that maximizes the number of host offspring (i.e. host fitness) because each offspring is a new habitat for colonization by the symbiont's propagules.

A significance of vertical transmission in reducing conflict was revealed by an important experiment conducted by Bull *et al.* (1991) on a bacterial virus, the filamentous f1 phage. This virus can not only be transmitted vertically by being integrated into the bacterial chromosome and copied passively with each round of bacterial chromosomal replication and cell division, but also horizontally, by release from bacterial cells. Two strains of f1 phage that differed in the extent to which they depressed the growth rate of the host *Escherichia coli* bacteria were used. They were grown together in competition on *E. coli*. When cultured under conditions that prevented horizontal transmission, the less costly viral strain increased in number relative to the more damaging strain, but when grown under conditions that permitted the production and release of phage progeny, this advantage was lost. These results demonstrate that vertical transmission selects for cooperative partners that impose low costs.

The relevance of these results to symbioses has been demonstrated for a number of associations. In particular, the acquisition of vertical transmission by endophytic fungi of grasses has played a crucial role in the evolution of symbionts with a protective role against herbivory from pathogens of grasses (Clay & Schardl, 2002). A further illustration of the importance of mode of transmission is provided by experiments that manipulated the mode of transmission of symbiotic algae *Symbiodinium* in the jellyfish *Cassiopeia xamachana* (Sachs & Wilcox, 2006). The starting material for the experiment was an alga-free culture of genetically identical host polyps and pooled culture of algal symbionts derived from a number of symbiotic *C. xamachana*. The algal culture was likely to include multiple genotypes that varied in their quality as symbionts. The experiment comprised two regimes. In the vertical transmission regime, a cohort of hosts was infected with the algal culture and then maintained through two asexual generations with vertical transmission. This regime was predicted to select for algae that promoted host fitness. In the horizontal transmission regime, algae expelled from the first cohort of infected hosts were administered to a new cohort of alga-free hosts over two consecutive generations. This regime was expected to select for infective algae. The symbiotic traits of the algae subjected to the two selection regimes were different. The vertically transmitted algae supported higher host growth and reproductive rates and released fewer algal cells available for horizontal transmission

Table 1 Impact of mode of transmission on the symbiotic traits of the alga *Symbiodinium* in the jellyfish *Cassiopeia xamachana* (data reproduced from Table 1 of Sachs & Wilcox (2006) expressed as mean and estimated SE)

| Transmission mode | Host growth rate (mg d ⁻¹) | Host reproductive rate (number of buds d ⁻¹) | Algal expulsion rate (number of cells h ⁻¹) |
|-------------------|--|--|---|
| Horizontal | 0.041 ± 0.005 | 1.44 ± 0.101 | 997 ± 227 |
| Vertical | 0.090 ± 0.004 <i>P</i> < 0.001 | 2.24 ± 0.163 <i>P</i> < 0.05 | 353 ± 88 <i>P</i> < 0.05 |

(Table 1). These results are consistent with the prediction that vertical transmission selects against cheating.

V. Discussion

Extant symbioses have persisted for a long time despite the occurrence of conflict and cheating. I have proposed in this article that tolerance of cheating over costly interactions might be promoted by benefit accruing from cost-free interactions (see Section III); and further research will be very valuable for assessing the significance of cost-free interactions in this context. More progress has been made on modes of conflict resolution (see Section IV), but much is still not understood. In particular, although vertical transmission appears to be an effective route to eliminate cheating, it is not adopted by many symbioses (Wilkinson & Sherratt, 2001). Why not? Vertical transmission may be disadvantageous, first, because the symbionts might interfere with host developmental processes or consume limiting host nutrients and, second, for hosts in variable environments where the genotypes of symbionts borne by the parents are not necessarily optimal for the offspring. For some types of symbiosis, vertical transmission may be precluded by anatomical constraints. For example, root symbionts of plants have no access to the shoot-borne reproductive organs and they are transmitted horizontally, but vertical transmission is the norm for several shoot-borne symbionts (e.g. cyanobacteria in *Azolla*; Lumpkin & Plucknett, 1980). Horizontal transmission at sexual reproduction of lichen fungi can also be linked to the production of the sexual spores in the cortex of the lichen thallus, spatially separate from the algal cells. Is cheating more widespread among symbioses with exclusive horizontal transmission than those with vertical transmission, and is coercion a more important mechanism for conflict resolution in horizontally than in vertically transmitted symbioses? At present, there are insufficient data to answer these intriguing questions.

In principle, conflict and cheating can overwhelm symbioses, leading to the evolutionary transition to parasitism, extinction of the symbiotic organisms, or reversion to the free-living condition (Sachs & Simms, 2006). The incidence of symbiosis breakdown can provide a first indication of the effectiveness of conflict resolution mechanisms in symbioses.

There are few unambiguous examples of parasitic species with symbiotic ancestors. Some cheating species (e.g. cheats of seed predator pollination systems, mycoheterotrophic plant parasites described already in this article) depend on the persistence of the symbiosis that they exploit. Symbiosis-derived parasites that are independent of related symbioses have evolved among lycaenid butterflies. The ancestral lycaenid condition was almost certainly in symbiosis with ants, which protect the caterpillars from predators. Nevertheless, an estimated 200 (4%) of the c. 5000 lycaenid species parasitize their ant partners, and the parasites have evolved independently several times, including within the *Maculinea-Phengaris* clade (Als *et al.*, 2004).

The various routes for conflict resolution described in this article probably play a major role in the rarity of symbiosis-derived parasitism. A further process may also occur for some types of symbiosis: that parasitic relationships evolve relatively frequently but they are evolutionarily unstable, with the partners either becoming extinct or reverting to the free-living condition. The incidence of extinction and transition to the free-living lifestyle in symbiotic lineages has not been studied in detail. Nevertheless, various phylogenetic studies have revealed nonsymbiotic taxa embedded within clades of symbiotic organisms. For some of these evolutionary transitions, the selection pressures are obscure. For example, it is not known why lichenized fungi have, on multiple occasions, reverted to the free-living habit (Lutzoni *et al.*, 2001). For other associations, plausible selection pressures can be invoked. Among the lycaenid butterflies, an estimated 25% of species are secondarily independent of ants; and they might have been 'abandoned' by their ant partners in response to parasitic tendencies of the lycaenid (Pierce *et al.*, 2002). Ant associations with homopteran insects are evolutionarily extremely labile, but the multiple gains and losses of the symbiosis can be linked more readily to the feeding habits of the homopteran than to conflict between the ants and homopterans. In aphids of the genus *Chaitophorus*, species with long mouthparts that feed from deep sieve elements in their host plants are vulnerable to predators because of the extended time required to withdraw their stylets; they are also particularly predisposed to form and retain associations with ants. By contrast, species with more shallow feeding sites derive relatively small or variable benefits of ant-tending and are

prone to losing the association. In these systems, the incidence of the symbiosis is driven by selection pressures on the plant utilization traits of the aphid (Shingleton *et al.*, 2005).

These considerations lead to two working hypotheses. The first is that extant symbioses are generally robust to cheats. Factors contributing to this condition probably include the selective advantage of cost-free interactions (which are not vulnerable to cheating) and effective mechanisms to suppress cheating. The second hypothesis is that, although among-partner conflict can influence the traits of organisms in symbiosis, it does not generally dictate the evolutionary fate of symbioses. The hypotheses are constructed from limited, often 'single-case' datasets and from plausible inference. They need to be tested systematically with a variety of symbiotic systems. The opening sentence of this article requires qualification. Things do fall apart but, for symbioses, not very often and not generally as a consequence of internal strife.

Acknowledgements

I thank the editors of *New Phytologist* for the invitation to write this review, Drs Martin Bidartondo and Rusty Rodriguez for valuable discussions, anonymous referees for helpful comments, and the Biotechnology and Biological Sciences Research Council for financial support.

References

- Als TD, Villa R, Kandul NP, Nash DR, Yen S-H, Hsu Y-F, Mignault AA, Roonsma JJ, Pierce NE. 2004. The evolution of alternative life histories in large blue butterflies. *Nature* 432: 386–390.
- Althoff DM, Segraves KA, Leebens-Mack J, Pellmyr O. 2006. Patterns of speciation in the yucca moths: parallel species radiations within the *Tegeticula yuccasella* species complex. *Systematic Biology* 55: 398–410.
- Axelrod R. 1984. *The evolution of co-operation*. New York, NY, USA: Basic Books Inc.
- Bidartondo MI, Redecker D, Hijri I, Wiemken A, Bruns TD, Dominguez L, Sérsic A, Leake JR, Read DJ. 2002. Epiparasitic plants specialised on arbuscular mycorrhizal fungi. *Nature* 419: 389–392.
- Bronstein JL. 2001. The exploitation of mutualisms. *Ecology Letters* 4: 277–287.
- Bryla DR, Eissenstat D. 2005. Respiratory costs of mycorrhizal associations. In: Lambers H, Ribas-Carbo M, eds. *Plant respiration. From cell to ecosystem*. Dordrecht, the Netherlands: Springer, 207–224.
- Bshary R. 2002. Biting cleaner fish use altruism to deceive image-scoring client reef fish. *Proceedings of the Royal Society of London Series B* 269: 2087–2093.
- Bshary R, Grutter AS. 2005. Punishment and partner switching cause cooperative behaviour in a cleaning mutualism. *Biology Letters* 1: 396–399.
- Bshary R, Grutter AS. 2006. Image scoring and cooperation in a cleaner fish mutualism. *Nature* 441: 975–978.
- Bull JJ, Molineux IJ, Rice WR. 1991. Selection of benevolence in a host–parasite system. *Evolution* 45: 875–882.
- Clay K, Schardl C. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist* 160: S99–S127.
- Conner RC. 1995. The benefits of mutualism: a conceptual framework. *Biological Reviews* 70: 427–457.
- Cook JM, Rasplus J-Y. 2003. Mutualists with attitude: coevolving fig wasps and figs. *Trends in Ecology and Evolution* 18: 241–248.
- Douglas AE. 1981. Uric acid utilisation in *Platymonas convolutae* and symbiotic *Convoluta roscoffensis*. *Journal of the Marine Biological Association UK* 63: 435–447.
- Douglas AE, Minto LB, Wilkinson TL. 2001. Quantifying nutrient production by the microbial symbiosis in an aphid. *Journal of Experimental Biology* 204: 349–358.
- Embley TM, Martin W. 2006. Eukaryotic evolution, changes and challenges. *Nature* 440: 623–630.
- Fenchel T, Finlay BF. 1995. *Ecology and evolution in anoxic worlds*. Oxford, UK: Oxford University Press.
- Ferriere R, Bronstein JL, Rinaldi S, Law R, Gauduchon M. 2002. Cheating and the evolutionary stability of mutualisms. *Proceedings of the Royal Society of London B* 269: 773–780.
- Fitter AH. 2006. What is the link between carbon and phosphorus fluxes in arbuscular mycorrhizas? A null hypothesis for symbiotic function. *New Phytologist* 172: 3–6.
- Gaume L, McKey D. 1999. An ant–plant mutualism and its host-specific parasite: activity rhythms, young leaf patrolling, and effects on herbivores of two specialist plant–ants inhabiting the same myrmecophyte. *Oikos* 84: 130–144.
- Izzo TJ, Vasconcelos HL. 2002. Cheating the cheater: domatia loss minimises the effects of ant castration in an Amazonian ant–plant. *Oecologia* 133: 200–205.
- Julou T, Burghardt B, Gebauer G, Berveiller D, Damesin C, Selosse MA. 2005. Mixotrophy in orchids: insights from a comparative study of green individuals and nonphotosynthetic individuals of *Cephalanthera damasonium*. *New Phytologist* 166: 639–653.
- Kiers ET, van der Heijden MGA. 2006. Mutualistic stability in the arbuscular mycorrhizal symbiosis: exploring hypotheses of evolutionary cooperation. *Ecology* 87: 1627–1636.
- Kiers ET, Rousseau RA, West SA, Denison RF. 2003. Host sanctions and the legume–*Rhizobium* mutualism. *Nature* 425: 78–81.
- Leake JR. 2004. Myco-heterotroph/epiparasitic plant interactions with ectomycorrhizal and arbuscular mycorrhizal fungi. *Current Opinion in Plant Biology* 7: 422–428.
- Letourneau DK. 1990. Code of ant–plant mutualism broken by parasite. *Science* 248: 215–217.
- Lodwig EM, Hosie AHF, Bordes A, Findlay K, Allaway D, Karunakaran R, Downie JA, Poole PS. 2003. Amino-acid cycling drives nitrogen fixation in the legume–*Rhizobium* symbiosis. *Nature* 422: 722–726.
- Lumpkin TA, Plucknett DL. 1980. *Azolla*: botany, physiology and use as a green manure. *Economic Botany* 34: 111–153.
- Lutzoni F, Pagel M, Reeb V. 2001. Major fungal lineages are derived from lichen symbiotic ancestors. *Nature* 411: 937–940.
- Maloof JE, Inouye DW. 2000. Are nectar robbers cheaters or mutualists? *Ecology* 81: 2651–2661.
- Marussich WA, Machado CA. 2007. Host-specificity and coevolution among pollinating and nonpollinating New World fig wasps. *Molecular Ecology* 16: 1925–1946.
- Modjo HS, Hendrix JW. 1986. The mycorrhizal fungus *Glomus macrocarpum* as a cause of tobacco stunt disease. *Phytopathology* 76: 688–691.
- Mooring MS, Mundy PJ. 1996. Interactions between impala and oxpeckers at Matobo National Park, Zimbabwe. *African Journal of Ecology* 34: 54–65.
- Mullins DE, Cochran DG. 1975. Nitrogen metabolism in the American cockroach. *Comparative Biochemistry and Physiology* 50A: 489–510.
- Muscatine L, Falkowski PG, Porter JW, Dubinsky Z. 1984. Fate of photosynthetic fixed carbon in light- and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. *Proceedings from the Royal Society of London B* 222: 181–202.
- Navarro L. 2000. Pollination ecology of *Anthyllus vulneraria* subsp. *vulgaris* (Fabaceae): nectar robbers as pollinators. *American Journal of Botany* 87: 980–985.
- Pellmyr O. 2002. Pollination by animals. In: Herrera CM, Pellmyr O, eds. *Plant–animal interactions*. Oxford, UK: Blackwell Science, 157–184.

- Pellmyr O, Leebens-Mack J. 2000. Reversal of mutualism as a mechanism for adaptive radiation in yucca moths. *American Naturalist* 156: S62–S76.
- Pellmyr O, Leebens-Mack J, Huth CJ. 1996. Non-mutualistic yucca moths and their evolutionary consequences. *Nature* 380: 155–156.
- Pierce NE, Braby MF, Heath A, Lohman DJ, Mathew J, Rand DB, Travassos MA. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annual Reviews of Entomology* 47: 733–771.
- Redecker, D., Kodner, R. Graham, L.E. 2002. *Palaeoglonius grayi* from the Ordovician. *Mycotaxon* 84: 33–37.
- Redman RS, Davigan DD, Rodriguez RJ. 2001. Fungal symbiosis from mutualism to parasitism: who controls the outcome, host or invader? *New Phytologist* 151: 705–716.
- Sachs JL, Mueller UG, Wilcox TP, Bull JJ. 2004. The evolution of cooperation. *Quarterly Review of Biology* 79: 135–160.
- Sachs JL, Simms EL. 2006. Pathways to mutualism breakdown. *Trends in Ecology and Evolution* 21: 585–592.
- Sachs JL, Wilcox TP. 2006. A shift to parasitism in the jellyfish symbiont *Symbiodinium microadriaticum*. *Proceedings of the Royal Society of London Series B* 273: 425–429.
- Shingleton AW, Stern DL, Foster WA. 2005. The origin of a mutualism: a morphological trait promoting the evolution of ant–aphid mutualisms. *Evolution* 59: 921–926.
- Simon L, Bousquet J, Levesque R, Lalonde M. 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature* 363: 67–69.
- Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*. 2nd ed. London, UK: Academic Press.
- Tanaka A, Christensen MJ, Takemoto D, Park P, Scott B. 2006a. Reactive oxygen species play a role in regulating a fungus–perennial ryegrass mutualistic interaction. *The Plant Cell* 18: 1052–1066.
- Tanaka Y, Miyajima T, Koike I, Hayashibara T, Ogawa H. 2006b. Translocation and conservation of organic nitrogen within the coral–zooxanthella symbiotic system of *Acropora pulchra*, as demonstrated by dual isotope-labeling techniques. *Journal of Experimental Marine Biology and Ecology* 336, 110–119.
- Taylor DL, Bruns TD. 1997. Independent, specialised invasions of the ectomycorrhizal mutualism by two non-photosynthetic orchids. *Proceedings of the National Academy of Sciences, USA* 94: 4510–4515.
- Trappe JM. 1987. Phylogenetic and ecological aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In: Safir CR, ed. *Ecophysiology of VA-mycorrhizal plants*. Boca Raton, FL, USA: CRC Press, 1–25.
- West SA, Griffin AS, Gardner A. 2007. Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. *Journal of Evolutionary Biology* 20: 415–432.
- Westerkamp C, Gottsberger G. 2000. Diversity pays in crop pollination. *Crop Science* 40: 1209–1222.
- Wilkinson DM, Sherratt TN. 2001. Horizontally acquired mutualisms, an unsolved problem in ecology? *Oikos* 92: 377–384.
- Woods R. 1999. *Reef evolution*. Oxford, UK: Oxford University Press.
- Yu DW, Pierce NE. 1998. Castration parasite of an ant–plant mutualism. *Proceedings of the Royal Society of London Series B* 265: 375–382.
- Zientz E, Dandekar T, Gross R. 2004. Metabolic interdependence of obligate intracellular bacteria and their insect hosts. *Microbiology and Molecular Biology Reviews* 68: 745–770.



About New Phytologist

- *New Phytologist* is owned by a non-profit-making **charitable trust** dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at www.newphytologist.org.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as-ready' via *OnlineEarly* – our average submission to decision time is just 28 days. Online-only colour is **free**, and essential print colour costs will be met if necessary. We also provide 25 offprints as well as a PDF for each article.
- For online summaries and ToC alerts, go to the website and click on 'Journal online'. You can take out a **personal subscription** to the journal for a fraction of the institutional price. Rates start at £135 in Europe/\$251 in the USA & Canada for the online edition (click on 'Subscribe' at the website).
- If you have any questions, do get in touch with Central Office (newphytol@lancaster.ac.uk; tel +44 1524 594691) or, for a local contact in North America, the US Office (newphytol@ornl.gov; tel +1 865 576 5261).