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Christie, P., Li, X. L., & Chen, B. D. (2004). Arbuscular mycorrhiza can depress translocation of zinc to shoots of host plants in soils moderately polluted with zinc. *Plant and Soil*, *261*(1-2), 209-217. https://doi.org/10.1023/B:PLSO.0000035542.79345.1b

Published in:
Plant and Soil

Plant and Soil **261:** 209–217, 2004.

Arbuscular mycorrhiza can depress translocation of zinc to shoots of host plants in soils moderately polluted with zinc

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Received 26 November 2002. Accepted in revised form 28 May 2003

Key words: arbuscular mycorrhiza, heavy metals, phytostabilization, soil pollution

Abstract

There is increasing and widespread interest in the maintenance of soil quality and remediation strategies for management of soils contaminated with organic pollutants and trace metals or metalloids. There is also a growing body of evidence that arbuscular mycorrhizal (AM) fungi can exert protective effects on host plants under conditions of soil metal contamination. Research has focused on the mechanisms involved and has raised the prospect of utilizing the mutualistic association in soil re-vegetation programmes. In this short paper we briefly review this research, summarize some recent work and highlight some new data which indicate that the alleviation of metal phytotoxicity, particularly Zn toxicity, by arbuscular mycorrhiza may occur by both direct and indirect mechanisms. Binding of metals in mycorrhizal structures and immobilization of metals in the mycorrhizosphere may contribute to the direct effects. Indirect effects may include the mycorrhizal contribution to balanced plant mineral nutrition, especially P nutrition, leading to increased plant growth and enhanced metal tolerance. Further research on the potential application of arbuscular mycorrhiza in the bioremediation or management of metal-contaminated soils is also discussed.

Introduction

It has been widely reported that ectomycorrhizal and ericoid mycorrhizal fungi can increase the tolerance of their host plants to heavy metals when the metals are present at toxic levels (Bradley et al., 1981, 1982; Jones and Hutchinson, 1988a,b). The underlying mechanism is thought to be the binding capacity of fungal hyphae to metals in the roots or in the rhizosphere which immobilizes the metals in or near the roots and thus depresses their translocation to the shoots (Bradley et al., 1981; Brown and Wilkins, 1985; Wasserman et al., 1987). However, no firm conclusions have been drawn concerning the role of AM fungi in plant uptake of trace metals (Zhu et al., 2001). Some workers have claimed that arbuscular mycorrhizas may also help plants to tolerate high levels of soil heavy metal contamination. For example, Heggo et al. (1990) and Hetrick et al. (1994) demonstrated that at high soil heavy metal concentrations, arbuscular mycorrhizal infection reduced the concentrations of Zn, Cd and Mn in plant leaves. However, there are few published reports describing the quantitative relationship between mycorrhizal infection and plant tolerance to heavy metal pollution under controlled conditions.

Trace metal contamination of soils can occur naturally from geological sources, for example Cu and Ni contamination of basaltic soils from the basalt parent

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material (Cruickshank, 1997), or can occur as a result of a wide range of industrial and agricultural activities. There is therefore increasing concern worldwide that trace metal pollution may harm human food safety and health, and there is much interest in the protection of unpolluted sites and the effective management of contaminated sites (Tordoff et al., 2000). Zinc is an essential plant nutrient and is one of the most ubiquitous trace metals in soils. When it is present in excessive concentrations it is toxic to plants and it is therefore often regarded as a potentially toxic element (Chaney, 1994).

Field investigations have indicated that mycorrhizal fungi can colonize plant roots extensively even in metal contaminated sites (Diaz and Honrubia, 1994; Gildon and Tinker, 1983; Pawlowska et al., 1996; Sambandan et al., 1992), and Zn- and Cd-tolerant fungal strains have been isolated from contaminated sites by several research groups (Hildebrandt et al., 1999; Kaldorf et al., 1999; Weissenhorn et al., 1994). These isolates have been used in laboratory studies in which they have been shown to be capable of increasing plant growth under artificial conditions of Zn contamination compared with reference strains. In fact, several studies have shown that fungi isolated from uncontaminated sources may also have the capacity to colonize host plants under conditions of moderate metal contamination (Liu et al., 2000; Tao, 1997). It can be concluded that tolerance to Zn contamination may commonly exist among arbuscular mycorrhizal fungi, but metal tolerance may be further enhanced when the fungi have adapted to high Zn concentrations.

Numerous experimental studies have indicated that under conditions of moderate Zn contamination, arbuscular mycorrhizal plants may exhibit much lower shoot concentrations of Zn and higher plant yields than non-mycorrhizal controls, indicating a protective effect of mycorrhizas on the host plants against potential Zn toxicity (Diaz et al., 1996; Dueck et al., 1986; Li and Christie, 2001; Liu et al., 2000). These observations have led to pot and laboratory experiments designed to elucidate possible mechanisms involved, such as metal tolerance of the mycorrhizal fungi, the effects of balanced mineral nutrition on Zn uptake by plants, factors affecting Zn uptake and partitioning in mycorrhizal plants, and the immobilization of metals in the roots of mycorrhizal plants and in the mycorrhizosphere. This paper summarizes some recent research on the effects of arbuscular mycorrhiza on Zn uptake and translocation by host plants growing in moderately contaminated soils or substrates. The ultimate aim is to utilize arbuscular mycorrhizas in their capacity to protect plants from Zn toxicity in the revegetation of moderately polluted soils. This may be achieved either by introducing effective fungal strains into the soil or by planting mycotrophic plant species and ensuring that the soil physico-chemical conditions are conducive to the development of arbuscular mycorrhizal associations.

Materials for the investigation of Zn toxicity

Field studies have been conducted on sites which have been subjected to anthropogenic inputs of metals, for example in biosolids applications or from mining activities, and soils collected from such contaminated sites have also been used in glasshouse experiments. Many of the glasshouse studies have been conducted using soils with low available P to maximize the capacity of the AM fungi to colonize the plant roots and to study the effects of added P on mycorrhizal plant growth and metal uptake. The experimental soils have often been spiked with Zn, usually followed by an equilibration period of several weeks to minimize artificially high bioavailability (and possibly toxicity) of the added Zn shortly after application. Experiments have often employed compartmented plant pots or 'rhizoboxes' in which the compartments have been separated by 30-μm mesh barriers that can be penetrated by extramatrical hyphae but not by plant roots. Thus, direct plant uptake of nutrients or metals by roots can be distinguished from uptake via external hyphae. The value of this technique, first devised by Schüepp et al. (1987) and modified for numerous subsequent studies, cannot be overestimated as it can overcome many of the problems arising from our inability to grow AM fungi in the absence of host plants. Harvesting of sufficient quantities of AM hyphae for chemical analysis is also difficult because the fungi cannot be grown in the absence of a host plant, and this has led to the development of methods such as the glass bead cultivation system (Chen et al., 2001; Redecker et al., 1995, 1998) in which host plants are grown in soil compartments, roots and associated AM hyphae can pass into compartments containing glass beads or quartz sand, and hyphae only can grow into central compartments containing glass beads only. Similarly, Joner et al. (2000) grew AM fungi in association with host plants in hyphal compartments containing sand within compartmented pots and recovered the excised hyphae for studies on the metal binding capacity of the hyphae. Tracer techniques have also been employed in a few studies. For example, Bürkert and Robson (1994) studied the uptake of ^{65}Zn by the hyphae of three AM fungi in association with subterranean clover and found that differences in their capacity to take up and transport Zn from root-free soil were related to the distances from the roots explored by the external hyphae of the fungi.

Development of mycorrhizal associations in Zn-contaminated soils

Glasshouse and laboratory experiments are open to the criticism that the degree of mycorrhizal colonization of the roots may be lower than in field conditions. Over a period of several years we have conducted a series of studies on the influence of AM fungi on Zn uptake by plants and have found a range of root colonization rates under different experimental conditions. However, in all the experiments a range of colonization rates of at least 30 – 50% occurred, and this did not appear to be changed by Zn additions (e.g. Chen et al., 2003). The fungi used were ubiquitous isolates and no attempt was made to select for isolates that had developed Zn tolerance. Zinc contamination therefore did not appear to have any notable effect on the establishment of the mycorrhizal association, even under relatively high rates of Zn contamination in our studies in which the plants often showed very poor growth compared with plants growing in uncontaminated control conditions. There have been several published reports of significant inhibition of mycorrhizal colonization by other trace metals such as Cu and Cd (Griffioen et al., 1994; Leyval et al., 1995). This apparent anomaly may be due to a relatively low physiological toxicity of Zn or a relatively high Zn tolerance among populations of AM fungi. It could be argued that in our experiments, particularly those using pots with separate compartments for plant growth and development of extramatrical hyphae, any negative effects of Zn on mycorrhiza establishment may have been masked by the application of fungal inoculum within a small volume of soil and roots. This deserves further investigation.

Influence of arbuscular mycorrhiza on plant growth

In general, mycorrhizal effects on plant growth have been related to the fertility of the soils used in experiments. Most investigators have selected soils of low fertility, especially soils with low available P. Moreover, in studies using compartment cultivation systems the plant roots have been restrained within a relatively small volume of growth substrate and this may have led to conditions of nutrient stress which may have exaggerated any mycorrhizal effects on plant growth. Under such conditions, the host plants might rely substantially on the mycorrhizal fungi for acquisition of mineral nutrients, and the growth promoting effects of mycorrhizal colonization on plants, especially higher shoot yields, have been highly significant as reported, for example by Chen et al. (2003). However, the differences in yield resulting from improved P nutrition of mycorrhizal plants compared with corresponding non-mycorrhizal controls would most likely conceal any direct involvement of mycorrhiza in alleviation of metal toxicity. To investigate the direct effects of mycorrhiza on metal uptake by plants, additional P fertilizers have been supplied to control plants in some studies to remove the P nutritional and growth promoting effects of the mycorrhiza (Li and Christie, 2001). An alternative strategy would be to use a soil of higher fertility, but this could lead to a marked reduction in mycorrhizal colonization rate.

Contribution of mycorrhiza to plant P nutrition

The mycorrhizal contribution to host plant P nutrition was confirmed in early studies (Baylis, 1959; Daft and Nicolson, 1966; Gerdemann, 1964), and this has long been accepted as the main beneficial effect of the association on host plants (Smith and Read, 1997). In compartment cultivation systems, extraradical hyphae increase the soil volume accessible for P acquisition (Li et al., 1991). In addition, P taken up is efficiently transported to plant roots (Cox et al., 1980; Smith and Smith, 1990). As a result, both the P concentration and P uptake of mycorrhizal plants can be much higher, sometimes several times higher, than those of corresponding controls (Chen et al., 2003). Even in pot experiments, in which both mycorrhizal and non-mycorrhizal roots may colonize the whole volume of substrate in the container, higher uptake rates of P by mycorrhizal plants have been observed.

High P uptake and transport efficiency seems to be an intrinsic attribute of mycorrhizal plants. Even when the P supply has been adequate for plant growth, the P concentration of xylem sap from mycorrhizal plants has been found to be significantly higher than that from non-mycorrhizal plants (unpublished data), but an excess supply of P does not produce any further plant growth increment.

Under conditions of Zn contamination, improved P nutrition might help the plant tolerate Zn toxicity through the resulting increase in plant growth. On naturally contaminated sites, lack of essential nutrients together with high metal concentrations have often been found to be the main obstacles to re-vegetation (Griffioen et al., 1994; Vangronsveld et al., 1996). We might therefore expect mycorrhizal plants to be more likely than non-mycorrhizal plants to colonize disturbed land because of the mycorrhizal contribution to plant mineral nutrition. In a survey of the mycorrhizal status of the 69 vascular plant species colonizing an area of Zn wastes in Poland, Gucwa-Przepiora and Turnau (2001) did indeed find that >60% of the plant species were mycorrhizal. However, two non-mycorrhizal species dominated the early successional part of the Zn heap where the only mycorrhizal species present showed no arbuscular development and the frequency of occurrence of individual AM species was highest on the oldest part of the area investigated. A strongly mycorrhizal plant species collected from the Zn wastes had abundant arbuscules except at the establishement of the vegetation (Turnau, 1998). This work indicates that non-mycorrhizal species may predominate during the initial colonization of contaminated sites and that mycorrhizal species may gradually become more important as succession proceeds.

Zinc uptake and partitioning in mycorrhizal plants

On soils without background contamination, improved plant Zn nutrition attributable to arbuscular mycorrhiza has been well documented in early studies (Dehn and Schüepp, 1989; Kothari et al., 1991). Dehn and Schüepp (1989) grew lettuce plants inoculated with one of three Glomus isolates in soils with high and low Zn and Cd levels. They found that mycorrhizal infection enhanced metal uptake by roots but not by shoots. In highly contaminated soils, metal concentrations were enhanced in the roots but decreased in the shoots compared with non-mycorrhizal plants. Retention of metals in the root systems was attributed to surface complexation of metals with cystein-containing ligands of fungal proteins and it was suggested that this may play a role in resistance of plants to heavy metals. Some studies have indicated that with increasing soil Zn contamination there may be a critical Zn concentration below which Zn uptake is enhanced while above this level there is inhibition of Zn translocation to the aerial parts of host plants (Chen et. al., 2003). In our recent series of experiments in which various rates of Zn (as Zn SO_4 solution) were applied to a calcareous soil without an equilibrium period prior to plant growth, the critical value consistently corresponded to about 50 mg kg^{-1} of added Zn (Chen et. al., 2003). Clearly, this critical value would need to be established more accurately for different soil types and plant species. The critical value could be readily determined using simple pot experiments under standard conditions, and the method used to determine soil available Zn would need to be standardized. Nevertheless, this may be an important concept because Zn is an essential nutrient for plant growth and at the same time a potentially toxic metal when excessive amounts are present.

Further studies on Zn partitioning in host plant shoots showed that a decrease in shoot Zn concentrations in mycorrhizal plants was mainly attributable to a decrease in foliar Zn. In contrast, the Zn concentrations in the stems showed no difference between mycorrhizal and non-mycorrhizal plants, and was sometimes slightly higher in mycorrhizal plants (X.L. Li and B.D. Chen, unpublished data). This may be interpreted as a compartmentation mechanism at the plant organ level for tolerating Zn toxicity. The negative effects of excessive Zn in plants may have been minimized by the arbuscular mycorrhiza.

If Zn was added only to the hyphal growth compartment in the compartment cultivation system and the plants therefore did not make direct contact with the added Zn, the decrease in the Zn concentration of plants tissues would be seen not only in the shoots, but also in the roots (Li and Christie, 2001). We suggest that in addition to their role in metal immobilization in the mycorrhizosphere, AM fungi may also act as an effective barrier controlling excessive Zn uptake into the root cells. Thus, the large amounts of Zn detected in roots in early studies would have been mainly immobilized in the apoplast. However, more detailed studies are needed on the physiological and chemical basis of the mechanisms involved.

Elemental interactions between Zn and P

It has been firmly established that there are interactions between Zn and P uptake and accumulation by plants, but the relationships are too complicated to allow simple generalizations, and when the effects of arbuscular mycorrhiza are superimposed the situation becomes even more complex (Lambert et al., 1979; Liu et al., 2000). Studies using low Zn soils have demonstrated greater plant yield and tissue Zn concentrations in mycorrhizal maize compared with mycorrhizal plants with no indication of nutrient interaction between Zn and P (Faber et al., 1990). Under some conditions uptake of both P and Zn can be stimulated simultaneously by mycorrhiza and under other conditions only P uptake is increased while Zn uptake is inhibited. The mycorrhiza may improve the balance of mineral nutrition, especially for trace nutrients. When these are in short supply their uptake may be stimulated, and when they are present in excess their uptake may be inhibited. Because mycorrhizal colonization is inextricably linked with P nutrition, the relationship between mycorrhizal infection and P nutrition cannot be ignored when effects of mycorrhiza on plant tolerance to elevated Zn are under consideration. Some studies have indicated that improved P nutrition which leads to increased plant growth can indirectly enhance plant tolerance to high Zn contamination. However, direct interactions between Zn and P may also occur during Zn uptake and transport processes in the mycorrhizal association. When no Zn is added to the soil, Zn^{2+} may be translocated via hyphae associated with the polyphosphate granules, but it is not clear how the metal would be translocated in contaminated soil. The P might precipitate Zn in hyphae or roots. Application of P fertilizer has been employed to alleviate negative effects of Zn contamination on plant growth, presumably through inhibition of Zn uptake by the additional phosphate. As pointed out by Shetty et al. (1995), Zn and P are mutually antagonistic when either element exceeds a threshold value. In addition to its effects on Zn uptake, P can also directly affect Zn detoxification in plants because phytic acid molecules are involved in Zn sequestration by some plant species (Van Steveninck et al., 1987). Interactions between Zn and P appear to be quite complex, and can occur throughout the soil-plant continuum. Further investigation into P–Zn interactions under a wide range of field conditions might help to elucidate the mechanisms of mycorrhiza-mediated alleviation of Zn toxicity.

Physical and chemical changes in the mycorrhizosphere

The extraradical hyphae of arbuscular mycorrhiza extend from roots to distant bulk soil, an effect equivalent to extending the volume of soil regarded as the rhizosphere and contributing to the mycorrhizal effect. Several recent studies on relationships between mycorrhiza and trace metals have shown a slight increase in soil pH in the mycorrhizosphere after plant growth (Unpublished data). Zinc mobility is greatly affected by soil pH. It is therefore quite possible that the immobilization of Zn by fungal activity is partly due to changes in soil pH and this could contribute to the inhibition of Zn uptake by mycorrhizal plants under conditions of high Zn contamination.

Li and Christie (2001) used soil moisture samplers to monitor changes in soil solution Zn concentrations and pH in the mycorrhizosphere of red clover. Soil solution Zn concentrations were lower and pH values were higher in mycorrhizal treatments than in non-mycorrhizal controls, and these trends were more pronounced at higher Zn application rates. The results also indicated that the protective effect of mycorrhiza against plant Zn uptake might have been associated with changes in Zn solubility mediated by changes in soil solution pH, or by immobilization of Zn in the extramatrical mycelium.

The protective role of mycorrhizal fungi in plant uptake of Zn

The mycelium of mycorrhizal fungi has been reported to possess very strong metal binding capacity. Joner et al. (2000) grew mycelium of several Glomus species in compartmented pots in association with subterranean clover or perennial ryegrass and investigated the binding of trace metals by excised mycelium incubated for 6 h in solutions containing metals. Metal sorption occurred within 30 min, appeared to be passive and was highest in a metal-tolerant isolate. Zinc sorption ranged from 5.6 to 76 mg g^{-1}, and was related to the equilibrium value in the solution. Using a modified glass bead compartment cultivation system, Chen et al. (2001) obtained enough fungal material to analyze the extraradical mycelium of *Glomus mosseae* and *G. versiforme* in association with maize and red clover. Although the study did not include elevated concentrations of Zn in the growth substrate, a very striking finding was that the Zn concentration in *G.*

mosseae was up to 1200 mg kg^{-1}, nearly 10 times higher than that of the corresponding roots. Such studies have provided strong evidence of a high affinity of AM fungi for Zn and other trace metals.

Biological characteristics of mycorrhizas, such as the distribution and quantity of internal hyphae, could have a strong influence on the effectiveness of the mycorrhizal barrier to Zn translocation by determining the surface area available for immobilization of metals in the roots. Turnau (1998) examined the localization of heavy metals within plant and fungal structures in *Euphorbia cyparissias* collected from sites including Zn wastes. Scanning electron microscopy with an energy dispersive X-ray microanalysis system showed that crystalloid depositions in cortical cells and around fungal hyphae contained higher concentrations of Zn than root cell wall and fungal structures. Chemical analysis of excised external mycelium is much easier to perform and showed that the Zn concentration in *G. mosseae* associated with maize roots was much higher than that in *G. versiforme* (Chen et al., 2001). Correspondingly, *G. mosseae* always produced extensive external hyphae but relatively few spores, while *G. versiforme* was sporiferous and produced sparse mycelium. Obviously the quantity of external mycelium and internal hyphae could be related to the metal binding capacity of mycorrhizal fungi and also the mycorrhizal effects in the rhizosphere, and as a result might help to determine to what extent mycorrhizas could enhance host plant tolerance to trace metal contaminants.

The metal binding capacity of excised mycelium was further studied in subsequent unpublished work in which mycelium of *G. mosseae* bound almost 3% of Zn on a dry matter basis, and the adsorption saturation point had still not been reached. In contrast, the Mn adsorption capacity of the mycelium was relatively weak. These results are consistent with earlier observations that plant Mn uptake was not diminished by mycorrhizal colonization (Posta et al., 1994), and lend support to the involvement of an immobilization mechanism in alleviation of Zn toxicity by mycorrhiza.

Some investigations have indicated that the Zn distribution pattern changed in mycorrhizal compared to non-mycorrhizal roots (Kaldorf et al. 1999). In mycorrhizas, Zn was located mainly in the arbuscules and other fungal structures such as internal hyphae. In contrast, in the corresponding roots, Zn was distributed in the outer layers. Clearly, establishment of a mycorrhizal association would greatly increase the metal binding capacity of roots, and this could explain the enhanced Zn accumulation in mycorrhizas and the depression of Zn translocation to the shoots.

Potential application of arbuscular mycorrhiza in remediation of Zn contaminated soils

Phytoremediation has been widely accepted as a safe, efficient and economical strategy for management of trace metal contaminated sites (Cunningham et al., 1995). The potential of AM fungi for phytoremediation of heavy metal polluted soils has recently been discussed by Leyval et al. (2002) and Turnau and Haselwandter (2002). Arbuscular mycorrhizas are likely most suitable for phytostabilization of potentially toxic elements in moderately polluted soils. Being strongly adsorbed and bound by mycorrhizal structures, metals in soils may be retained within a certain volume of soil, with minimization of leaching processes and restriction of the zone of contamination, and plants may be protected from metal toxicity and environmental stress. Application of arbuscular mycorrhizas offers potential advantages for re-vegetation of polluted sites and restoration of disturbed ecosystems (Shetty et al., 1994). Mycorrhizal fungi appear to at least partially protect plants against the toxicity of trace metals and the host plant may give the fungus a selective survival advantage at a contaminated site (Donnelly and Fletcher, 1994). In addition, in view of the poor fertility and unbalanced mineral nutrient supply of many contaminated sites, application of AM fungi would be even more useful. In lightly contaminated agricultural soils, introduction of mycorrhizal associations into the planting system could possibly maintain soil productivity in sustainable systems. Reduced shoot metal accumulation could help safeguard components of the human food chain such as cereal grains. There may even be some possibility of using AM fungi to enhance phytoextraction of soil metals (Ernst, 2000). In this connection, it is interesting to note that potentially mycorrhizal crop species such as barley can extract Zn from soil as efficiently as non-mycorrhizal plant species that have been used for phytoextraction such as *Brassica juncea* (Ebbs and Kochian, 1998). Application of mycorrhizas could thus become an important component of crop management in the future, especially in the development of low-input systems in which soil available P levels are not high enough to depress mycorrhizal colonization of the plant roots.

Further research

The protective effect of arbuscular mycorrhizas might be improved by screening for metal tolerant strains (Leyval et al., 1997) and using these to inoculate crop plants. Furthermore, we have used alkaline or neutral soils in our studies, which should be extended to include acid soils in which the mycorrhizal effects might be very different, especially as soil pH has large effects on metal mobility and bioavailability. We also need to investigate further the chemical and physiological processes occurring during metal immobilization in the rhizosphere, binding by mycorrhizal structures, and possible immobilization in the host plant roots. More field studies are required on the application of arbuscular mycorrhizas in remediation strategies for re-vegetation programmes in disturbed sites and the restoration of degraded soils (Turnau and Haselwandter, 2002).

Conclusions

Based on current experimental data, binding of trace metals by arbuscular mycorrhizal fungi seems likely to be the most important mechanism involved in the alleviation of Zn phytotoxicity by these fungi, and the significance of AM fungi in metal adsorption processes should be emphasized. The metal binding capacity of mycorrhizal fungi is so striking that both uptake of metals from soils and subsequent translocation to plant shoots may be effectively restricted under conditions of moderate pollution. The indirect involvement of arbuscular mycorrhiza in alleviation of Zn toxicity may also be important under certain conditions. Balanced mineral nutrition, especially enhanced P uptake, is likely to be very important for plant growth in the use of phytostabilization at many sites that are moderately polluted by trace metals. Phytoextraction of lightly contaminated agricultural soils of low available P status by mycorrhizal cereal crops may also be possible.

Acknowledgements

We thank the Major State Basic Research Development Program of the People's Republic of China (Project G1999011807), the National Natural Science Foundation of China (Project 40071050), the British Council (Project PEK/0992/314) and the Royal Society of London for generous financial support and two anonymous referees for their valuable comments on the manuscript.

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