26. Nosé, S. A molecular dynamics method for simulations in the canonical ensemble. *Mol. Phys.* **52,** 255–268 (1984).
27. Wallace, D. C. *Thermodynamics of Crystals* (Dover Publications, New York, 1998).

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Major fungal lineages are derived from lichen symbiotic ancestors

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About one-fifth of all known extant fungal species form obligate symbiotic associations with green algae, cyanobacteria or with both photobionts. These symbioses, known as lichens, are one way for fungi to meet their requirement for carbohydrates[1,2]. Lichens are widely believed to have arisen independently on several occasions, accounting for the high diversity and mixed occurrence of lichenized and non-lichenized (42 and 58%, respectively) fungal species within the Ascomycota[3,4]. Depending on the taxonomic classification chosen[2,5,6], 15–18 orders of the Ascomycota include lichen-forming taxa, and 8–11 of these orders (representing about 60% of the Ascomycota species) contain both lichenized and non-lichenized species. Here we report a phylogenetic comparative analysis of the Ascomycota, a phylum that includes greater than 98% of known lichenized fungal species[5]. Using a Bayesian phylogenetic tree sampling methodology[7,8] combined with a statistical model of trait evolution[9], we take into account uncertainty about the phylogenetic tree and ancestral state reconstructions. Our results show that lichens evolved earlier than believed, and that gains of lichenization have been infrequent during Ascomycota evolution, but have been followed by multiple independent losses of the lichen symbiosis. As a consequence, major Ascomycota lineages of exclusively non-lichen-forming species are derived from lichen-forming ancestors. These species include taxa with important benefits and detriments to humans, such as *Penicillium* and *Aspergillus*[10–12].

To investigate the evolution of the lichen symbiosis it is necessary to account for the phylogenetic relationships within the Ascomycota, and to infer the rates and likely pattern of gains and losses of the symbiotic state. We reduced the high level of uncertainty associated with small subunit nuclear ribosomal RNA gene (SSU nuclear rDNA) phylogenies of the Ascomycota[13–15], by obtaining sequences from the small and large subunit (LSU) of the nuclear rRNA genes for 52 species of the Ascomycota. Our sample includes representatives from 24 of 46 orders[2], representing ≈75% of the Ascomycota species diversity.

Phylogenetic comparative investigations typically rely on a single phylogenetic tree and reconstruct ancestral states on the basis of the method of parsimony. However, phylogenetic trees are rarely known without error and different tree topologies can give different estimates of ancestral states. In addition, ancestral states reconstructed by parsimony do not account for the statistical uncertainty of ancestral inferences. Both of these problems are acute when reconstructing the evolution of the lichen symbiosis. All previous broad phylogenetic studies of the Ascomycota had low bootstrap support and unstable relationships in critical portions of the trees[4,12–17]. Furthermore, parsimony methods may perform poorly when rates of character evolution are high[18,19].

We used a Bayesian statistical procedure based on Markov chain Monte Carlo (MCMC) sampling methods[7] to account for phylogenetic uncertainty. This sampling procedure allows us to draw a random sample from the universe of possible phylogenetic trees. The frequency distribution of the sample estimates the posterior probability distribution of trees (see Methods). From the distribution of sampled trees we calculated the posterior probability of ancestral nodes and focused our data interpretation on those nodes with the highest statistical certainty.

We used a statistical model of trait evolution[9,20,21] to estimate on each tree the evolutionary rate of gains and losses of lichenization, and the most probable ancestral states (lichen-forming/non-lichen-forming) at specified nodes. Rates of evolution between states are calculated over all possible states at each node of a given tree and are therefore independent of any particular reconstruction of the ancestral states. The model of trait evolution takes into account the lengths of the branches of the phylogenetic tree, does not constrain the rates of gains and losses *a priori*, and expresses the statistical uncertainty associated with estimates of ancestral states at each node. To derive an overall estimate of the rates of evolution or the probability of an ancestral state, the estimates from each tree are averaged (see Methods). Our inferences about the nature of the evolutionary processes underlying lichen evolution thereby take account of uncertainty inherent to the phylogenetic hypothesis, and are not conditional on any particular tree.

We sampled 19,900 phylogenetic trees using the MCMC procedure[7], and estimated by maximum likelihood the rates of gains and losses of lichenization on each (Fig. 1). Larger rate values correspond to a higher expected number of transformations (losses or gains), and the loss/gain ratio (dots in Fig. 1) directly estimates the ratio of expected losses to expected gains of lichenization during evolution.

In contrast with previous work on the evolution of the lichen symbiosis[4], our results show that rates of loss of lichenization exceed rates of gain in the Ascomycota. In 18,029 of the 19,900 sampled trees (90.6%) the estimated rate of loss exceeds the rate of gain (that is, the loss/gain ratio is greater than one, and therefore is above the

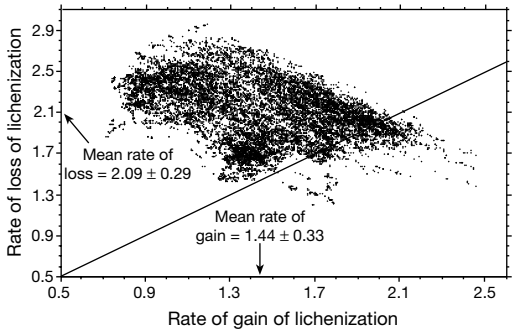

Figure 1 The rate of loss of lichenization exceeds the rate of gain of lichenization, independently of tree topology. Data are for 19,900 MCMC trees. The 1:1 relationship is indicated by the solid line.

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diagonal line in Fig. 1). The average loss/gain ratio is 1.56 ± 0.53 and is skewed towards higher ratios (range = 0.56–3.24). Across trees the highest rates of loss are associated with the lowest rates of gain (Fig. 1; $r = -0.40$). The ratio of losses to gains is, however, independent of the phylogenetic tree topology (correlation between ratio of rate of losses to gains and log likelihood of trees = –0.024).

These results indicate that there have been at least 1.5 times as many losses of the lichen symbiotic state as gains during the evolution of the Ascomycota. The majority rule consensus phylogenetic tree (Fig. 2, right) shows the most probable occurrence of gains and losses of lichenization and their phylogenetic context. We show this tree not to propose a particular topology, but principally

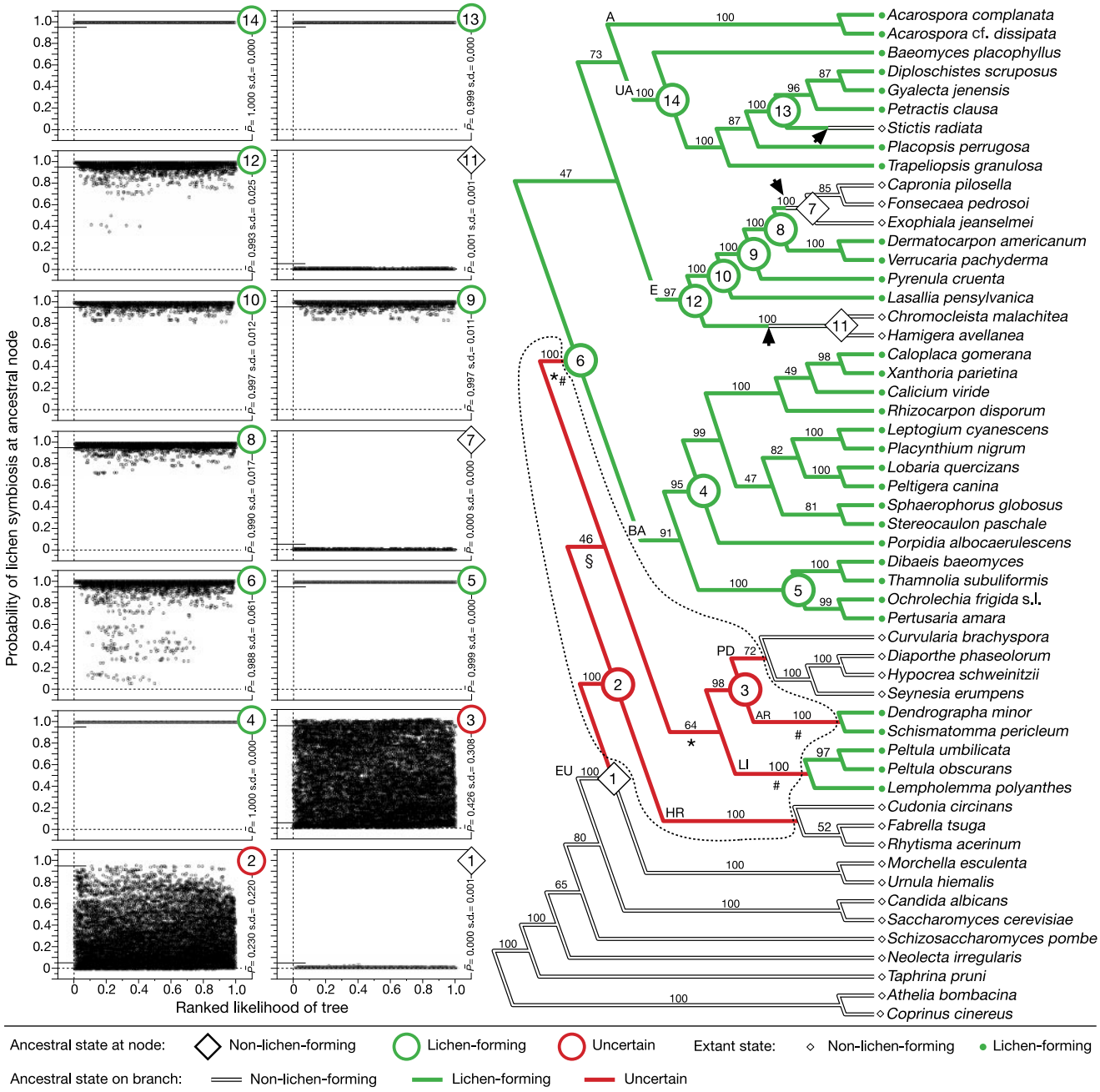

Figure 2 Bayesian posterior probabilities for reconstructed evolution of the lichen symbiosis and for each node of the Ascomycota phylogeny. Numbers (1–14) connect nodes in the tree with their respective graphs. Left, reconstructed probability (Discrete[9,20]) that ancestral state was lichen-forming at specified node on 19,900 trees generated by MCMC sampling (BAMBE[7]). The average probability and standard deviation as calculated across all trees is provided on the *y*-axis to the right of each graph. Right, Ascomycota majority rule consensus of 19,900 MCMC-sampled trees on the basis of SSU and LSU nuclear rDNA sequences. The number above each branch corresponds to the posterior probability (%) of the node to which it points. The region of the tree for which the ancestral states of branches could not be extrapolated, because of uncertainty associated with specific nodes, is indicated by a dotted line. Arrows indicate losses of lichenization. The non-lichenized Archiascomycetes (subphyllum Taphrinomycotina) are represented by the paraphyletic *Taphrina*, *Schizosaccharomyces* and *Neolecta*. The non-lichenized Hemiascomycetes (Saccharomycotina), are represented by *Saccharomyces* and *Candida*. The operculate discomycetes (Pezizomycotina), represented by *Urnula* and *Morchella*, form the first (basal) divergence within the highly stable Euascomycetes (EU). The Acarosporaceae (A), 'unitunicate ascohymenials' (UA), 'bitunicate ascohymenials' (BA) and 'Eurotiomycotina' (E) consistently formed a monophyletic group—the 'Lecanoromycotina' (node 6). The Helotiales–Rhytismatales (HR), Lichinales (LI), Arthoniales (AR) and Pyrenomycetes–Dothideales (PD) appear as a paraphyletic assemblage with poor support.

to provide a means for discussing central events of evolution in the Ascomycota. The numbers above each internal branch of the consensus tree (Fig. 2) correspond to the Bayesian posterior probability of the node to which a branch points. Nodes with values of 100 define a collection of species all of which—and only those of which—appeared in every one of the trees sampled from the Markov chain. Other nodes were less certain, and emphasize the need for a statistical approach.

We restricted our reconstructions[9] of the probable ancestral states (lichen-forming/non-lichen-forming) to fourteen nodes, each of which gained a posterior probability of $\geq 95\%$ in our MCMC sample (numbers 1–14; Fig. 2, right). These nodes delineate groups of lichen-forming and non-lichen-forming species in such a way as to make it possible to put reasonable upper and lower limits on the number of independent gains and losses of lichenization. The left panel of Fig. 2 plots the probability of the ancestral state for each of these labelled nodes as reconstructed across the 19,900 sampled trees.

The ancestor at 9 of the 14 nodes is lichenized (green circles, corresponding to >0.98 posterior probability of reconstructed state; Fig. 2), 3 nodes are non-lichenized (black diamonds, corresponding to <0.01 posterior probability), and the symbiotic status for two ancestral nodes is uncertain (red circles, corresponding to a 0.23 and 0.43 probability of being lichen-forming). Thus, green areas of the tree are regions of lichenization, red areas are regions in which the ancestral state is uncertain, and the remaining (uncoloured) branches correspond to non-lichenized regions.

The pattern of ancestral states we find can be used to infer that a minimum of one and a maximum of three gains of lichenization occurred during the evolution of the Ascomycota, with perhaps one or two being the most likely. If lichen formation originated immediately after node 2 (branch labelled with §), then one gain of lichenization is implied for the Ascomycota. Two origins of lichen symbiosis are implied (branches labelled with *) if lichenization evolved independently at node 6 and again at the base of the clade that includes the Lichinales (LI), Arthoniales (AR) and Pyrenomycetes–Dothideales (PD). Three independent gains are implied if the closely related AR and LI groups independently evolved lichenization (branches labelled with #).

By comparison, a minimum of three and possibly four losses of lichenization have occurred in the Ascomycota. Nodes 8, 12 and 13 each have very high posterior probabilities on the tree (Fig. 2, right), each is reconstructed to have a high posterior probability of being lichenized (Fig. 2, left), and each is followed by a loss of lichenization on the tree. The non-lichenized members of the Ostropales (such as *Stictis radiata*, derived from node 13), the Chaetothyriales (all species derived from node 7), and the Eurotiales (node 11, which now includes the Onygenales[10,12]) are therefore all secondarily non-lichenized, being independently derived from lichenized ancestors. The Eurotiales and Onygenales together encompass about 320 species, none of which are lichenized[2]; the Chaetothyriales (≈ 75 species) are not known to have lichenized species; and the Ostropales (including Graphidales) comprise about 1,850 species, 10% of which are non-lichenized (≈ 180 species). A fourth loss of lichenization is implied at the base of the PD group if lichen formation indeed originated only once or twice within the Ascomycota (that is, at the branches labelled with § or with *; Fig. 2).

These results reshape in three related and important ways our understanding of the evolution of the lichen symbiosis in the Ascomycota. First, we find that lichens arose much earlier (node 6; Fig. 2) than previously thought. This suggests that the lichen symbiosis has been a relatively long standing relationship, and consequently, has had a larger role in the evolution of the Ascomycota than previously believed[4]. Second, several major lineages of strictly non-lichenized species (Chaetothyriales and Eurotiales) unexpectedly turn out to be derived from lichen-forming ancestors. If Fig. 2 is extrapolated to all Ascomycota species, then a minimum of about 4% of known extant non-lichenized Ascomycota species are secondarily derived from lichen symbiotic ancestors. The actual percentage could be much higher, as the non-lichenized species within the Arthoniales (AR) and Pyrenomycetes–Dothidiales (PD) also may have resulted from an ancestral loss of lichenization.

A third implication of our results is to emphasize the distinction between secondarily derived and primary non-lichenized Ascomycota. Candidiasis, for example, is caused by the primary non-lichenized fungus *Candida albicans*. A large number of Ascomycota species of relevance to humans, however, are shown here to be those that have secondarily lost the ability to form a lichen symbiosis. The Eurotiales (node 11), for example, include many of the most beneficial and detrimental fungi to humans because of their production of antibiotics and mycotoxins[11]. *Penicillium* is a member of the secondarily derived non-lichenized Eurotiales. Aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* (also members of the Eurotiales) on various nuts and grains are among the most potent carcinogenic compounds known[11]. Other attributes of members of the Eurotiales include being used in the ripening of bleu cheeses, being pathogens of citrus fruits, and being infective agents of animal and human diseases such as aspergilloses, caused by the secondarily derived non-lichenized *Aspergillus*[11]. The Herpotrichiellaceae (Chaetothyriales) is another example of a major fungal lineage derived from a lichenized ancestor that gave rise to opportunistic pathogens to humans. *Fonsecaea pedrosoi* (node 7; Fig. 2, right) is one of the causative agents of chromoblastomycosis, and certain species of *Exophiala* are pathogens of fish, including salmon[11]. Better insight into animal and human diseases caused by fungi may be gained by calling attention to the distinction between secondarily derived and primary non-lichen-forming taxa.

Secondarily derived non-lichen-forming fungi may be more likely to synthesize compounds that have medicinal, pharmacological, carcinogenic and food production properties because the original transition to lichenization may have shifted the selective pressure on biosynthetic pathways towards an accelerated origin of new secondary compounds. It is well known that lichen-forming Ascomycota are prolific in the production of unique secondary compounds (especially depsides and depsidones), some of which have antibiotic or anti-tumour properties[22]. It is expected that when lichen formation is lost, some of the genes involved in these biosynthetic pathways may be diverted to new functions in the secondarily derived non-lichen-forming organism. Phylogenies can help to identify additional species with possible benefits to humans.

Lichenicolous fungi (fungi dwelling on or in lichens as parasites, commensals or saprobes[2]) may provide one explanation for the high rate of loss of the lichen symbiotic habit reported here (Figs 1 and 2). Morphological evidence supports the hypothesis that many of these lichenicolous fungi are derived from lichen ancestors[23]. By shedding their lichenized habit and colonizing lichens, these non-lichenized fungi continue to obtain directly, or indirectly from the lichenized fungus, carbohydrates generated by the alga/cyanobacterium without having to find a specific free living photobiont with which to form a lichen thallus *de novo* each generation[23]. This transformation from a lichenized to a non-lichenized lichenicolous state may act as a fungal 'half-way house' that could facilitate further transitions to different substrates. The high rate of loss of lichenization that we have found corroborates a study that mutualism is not necessarily at the end of a unidirectional evolution from parasitism to mutualism[24].

A phylogenetic study of the basidiomycete *Omphalina*, which includes a mixture of closely related lichenized and non-lichenized species[25], and where a single unequivocal gain of lichenization was detected[26], supports the low rate of gains of lichenization reported here. We have shown elsewhere[27] that this specific transition to mutualism leads to accelerated rates of evolution at the molecular level. Slow growth in axenic culture of these lichen-forming mushrooms, the unusual high frequency of uninucleated individuals, and

the highly variable number of spores/basidium compared with their non-lichenized sister species, are further evidence that a high level of stress on the fungus is associated with a transition to the lichen symbiosis[28]. This may suggest that many attempts to form stable lichen symbioses occur in nature, but only rarely does a specific fungal lineage have all the requirements to survive the costs associated with a transition to this symbiotic state. □

Methods

DNA sequencing, taxon and character sampling

Total DNA was isolated, and the SSU and LSU nuclear rDNA were amplified, sequenced and aligned as described in ref. 16. Regions of the alignments where the placements of gaps were ambiguous were removed from the MCMC phylogenetic tree sampling analyses. Taxa were selected to represent all main ascomycete orders[2] known to include lichenized species (13 out of 15 orders) and nearly all main orders of Ascomycota known to include only non-lichenized species (11 out of 31 orders). At least 16 of the unsampled non-lichenized orders almost certainly fall entirely within existing non-lichenized clades (Fig. 2) and their sampling will not affect the results presented here. This is because the reconstruction of ancestral states is not weighted by the number of descendant taxa that have a particular state; rather, the reconstructed state depends on the relative frequencies of the states in the descendants and their phylogenetic distribution. If all of a group of unsampled taxa share a most recent common ancestor and the same state with a species already included in our study, our reconstructions are unchanged. Basidiomycota (*Athelia bombacina* and *Coprinus cinereus*) sequences were included as outgroups. (The voucher/GenBank information is available as Supplementary Information.) We generated a total of 20 SSU and 24 LSU nuclear rDNA sequences in this study. All these sequences were deposited in GenBank under accession numbers AF356653–AF356696. Ten SSU and 20 LSU sequences were from ref. 16, and the remaining 24 SSU and 10 LSU sequences were from GenBank.

MCMC phylogenetic tree sampling

We used MCMC methods[7] within a Bayesian framework to estimate the posterior probability of phylogenetic trees. The MCMC procedure ensures that trees are sampled in proportion to their probability of occurrence under the model of gene-sequence evolution. We generated 200,000 phylogenetic trees using the MCMC procedure, sampling every tenth one to assure that successive samples were independent[7,29]. We then removed the first 100 trees in the sample to avoid including any trees that might have been sampled before convergence of the Markov chain. We used the general time-reversible model of gene-sequence evolution combined with gamma rate heterogeneity to estimate the likelihood of each tree[30]. Information on the state of each species (lichen-forming/non-lichen-forming) was excluded from the MCMC sampling procedure to ensure that the distribution of tree topologies was not influenced by this trait. A series of runs using the BAMBE[7] 'global' and 'local' options was conducted to ensure that the Markov chain converged to the same region in the universe of trees.

Reconstruction of gains and losses, and ancestral states

We used a continuous time Markov model of trait evolution, as implemented in the computer program Discrete (available from M.P.), allowing independent gains and losses in each branch of the phylogenetic tree[20]. Parameters specifying rates of gain (q_{01}) and loss (q_{10}) of lichenization were calculated separately for each tree sampled in the MCMC procedure, following procedures in ref. 9. Because trees are represented in the sample in proportion to their likelihood, investigating the rates over all trees automatically weights our results by the likelihood of a particular tree type. A detailed description of the analyses performed for this study will be published as a book chapter (M.P. and F.L., manuscript in preparation).

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1. Hawksworth, D. L. The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycol. Res.* **95,** 641–655 (1991).
2. Hawksworth, D. L., Kirk, P. M., Sutton, B. C. & Pegler, D. N. *Dictionary of the Fungi* (CAB, Wallingford, 1995).
3. Barinaga, M. Origins of lichen fungi explored. *Science* **268,** 1437 (1995).
4. Gargas, A., DePriest, P. T., Grube, M. & Tehler, A. Multiple origins of lichen symbioses in fungi suggested by SSU rDNA phylogeny. *Science* **268,** 1492–1495 (1995).
5. Honegger, R. in *Lichen Biology* (ed. Nash, T. H.) 24–36 (Cambridge Univ. Press, Cambridge, 1996).
6. Hafellner, J. in *Handbook of Lichenology* Vol. 3 (ed. Galun, M.) 41–52 (CRC, Boca Raton, 1988).
7. Larget, B. & Simon, D. L. Markov chain monte carlo algorithms for the Bayesian analysis of phylogenetic trees. *Mol. Biol. Evol.* **16,** 750–759 (1999).
8. Huelsenbeck, J. P., Rannala, B. & Masly, J. P. Accomodating phylogenetic uncertainty in evolutionary studies. *Science* **288,** 2349–2350 (2000).
9. Pagel, M. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Syst. Biol.* **48,** 612–622 (1999).
10. Berbee, M. L., Yoshimura, A., Sugiyama, J. & Taylor, J. W. Is *Penicillium* monophyletic? An evaluation of phylogeny in the family Trichocomaceae from 18S, 5.8S, and ITS ribosomal DNA sequence data. *Mycologia* **87,** 210–222 (1995).
11. Alexopoulos, C. J., Mims, C. W. & Blackwell, M. *Introductory Mycology* (John Wiley & Sons, New York, 1996).
12. Suh, S.-O. & Blackwell, M. Molecular phylogeny of the cleistothecial fungi placed in Cephalothecaceae and Pseudeurotiaceae. *Mycologia* **91,** 836–848 (1999).
13. Berbee, M. L. & Taylor, J. W. From 18S ribosomal sequence data to evolution of morphology among the fungi. *Can. J. Bot.* (Suppl. 1) **73,** S677–S683 (1995).
14. Spatafora, J. W. Ascomal evolution of filamentous ascomycetes: evidence from molecular data. *Can. J. Bot.* (Suppl. 1) **73,** S811–S815 (1995).
15. Berbee, M. L., Carmean, D. A. & Winka, K. Ribosomal DNA and resolution of branching order among the Ascomycota: how many nucleotides are enough? *Mol. Phyl. Evol.* **17,** 337–344 (2000).
16. Bhattacharya, D. *et al.* Widespread occurrence of spliceosomal introns in the rDNA genes of ascomycetes. *Mol. Biol. Evol.* **17,** 1971–1984 (2000).
17. Liu, Y. J., Whelen, S. & Hall, B. D. Phylogenetic relationships among ascomycetes: evidence from the RNA polymerase II subunit. *Mol. Biol. Evol.* **16,** 1799–1808 (1999).
18. Collins, T. M., Wimberger, P. H. & Naylor, G. J. P. Compositional bias, character-state bias, and character-state reconstruction using parsimony. *Syst. Biol.* **43,** 482–496 (1994).
19. Maddison, D. R. Phylogenetic methods for inferring the evolutionary history and process of change in discretely valued characters. *Ann. Rev. Entomol.* **39,** 267–292 (1994).
20. Pagel, M. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc. R. Soc. Lond. B* **255,** 37–45 (1994).
21. Pagel, M. Inferring the historical patterns of biological evolution. *Nature* **401,** 877–884 (1999).
22. Elix, J. A. in *Lichen Biology* (ed. Nash, T. H.) 154–180 (Cambridge Univ. Press, Cambridge, 1996).
23. Rambold, G. & Triebel, D. The inter-lecanoralean associations. *Bibliotheca Lichenologica* **48,** 1–201 (1992).
24. Hibbett, D. S., Gilbert, L.-B. & Donoghue, M. J. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature* **407,** 506–508 (2000).
25. Lutzoni, F. & Vilgalys, R. *Omphalina* (Basidiomycota, Agaricales) as a model system for the study of coevolution in lichenized fungi. *Crypt. Bot.* **5,** 82–97 (1995).
26. Lutzoni, F. Phylogeny of lichen- and non lichen-forming omphalinoid mushrooms and the utility of testing for combinability among multiple data sets. *Syst. Biol.* **46,** 373–406 (1997).
27. Lutzoni, F. & Pagel, M. Accelerated evolution as a consequence of transition to mutualism. *Proc. Natl Acad. Sci. USA* **94,** 11422–11427 (1997).
28. Kranner, I. & Lutzoni, F. in *Plant Responses to Environmental Stresses: From Phytohormones to Genome Reorganization* (ed. Lerner, H. R.) 591–628 (Marcel Dekker, New York, 1999).
29. Wilson, I. & Balding, D. Genealogical inference from microsatellite data. *Genetics* **150,** 499–510 (1998).
30. Hillis, D. M., Moritz, C. & Mable, B. K. *Molecular Systematics* 2nd edn (Sinauer, Sunderland, 1996).

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Phylogenetic analyses do not support horizontal gene transfers from bacteria to vertebrates

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Horizontal gene transfer (HGT) has long been recognized as a principal force in the evolution of genomes[1]. Genome sequences of Archaea and Bacteria have revealed the existence of genes whose similarity to loci in distantly related organisms is explained most parsimoniously by HGT events[2–4]. In most multicellular organisms, such genetic fixation can occur only in the germ line. Therefore, it is notable that the publication of the human genome reports 113 incidents of direct HGT between bacteria