

Phylogeny, Natural Selection and the State of Mushroom Classification

Todd W. Osmundson, PhD

Department of Environmental Science, Policy & Management and Berkeley Natural History Museums

University of California, Berkeley, CA 94702, U.S.A.

email: toddo@berkeley.edu

ABSTRACT

Mushroom classification is currently undergoing a shift of historic proportions as analyses of DNA sequence data are revealing the need for an extensive overhaul of the Friesian classification system. In some cases the resulting rearrangements represent the progression of science aided by new types of data; in others, they remedy previous classifications that were based on scant evidence. Regardless of the type of data used, the primary goal of a scientific classification system is the same: a classification of organisms that reflects their underlying pattern of genetic relatedness. Founded on this premise, this paper addresses several topics relevant to mushroom classification: (1) the question of whether the “traditional” (i.e., comparative morphological) system of classifying fungi is fundamentally unscientific, or whether its principles have simply often been subjectively applied; (2) the pitfalls inherent in classifying organisms based on hypotheses related to the action of natural selection; (3) the advantages to combining both molecular and morphological approaches to classification; and (4) the question of whether DNA studies offer the solution to the problem of unstable classifications and the frequent name changes that follow from them. The most promising path to achieving a classification system that reflects underlying evolutionary relationships is to collect and analyze many data of various kinds, to incorporate new types of data as they become available, and to recognize that the stability of a classification is not an adequate goal in itself. The extinction of species poses a serious problem for efforts to reconstruct the fungal evolutionary tree, and as-yet-undiscovered contemporary taxa also contain clues that could aid in these efforts; therefore, the exploration and conservation of existing mushroom habitats are critical endeavors.

INTRODUCTION

As an increasing number of DNA-based studies examine the evolutionary relationships between fungi, it is no longer surprising to see morphological classifications overturned on genetic grounds. In some cases, these rearrangements offer new insights into evolutionary history – one example is the splitting of *Coprinus* into three distinct evolutionary lineages (Hopple & Vilgalys, 1999). In other cases, they reveal the fragile, and often subjective, placement of species in groups based on few characteristics, with placements changing based on differences in the opinions of different mycologists as to which characters best represent evolutionary history – one example is the shifting taxonomic position of the bolete now known as *Bothia castanella*, which has previously been placed in seven different genera (Halling et al., 2007). An article by Michael Kuo in a recent issue of *Mcllvainea* (Kuo, 2007) addressed this fundamental problem of subjective classification, and raised the thought-provoking question of whether mushroom classification is based on a weak foundation of poorly-formed hypotheses about insignificant character differences. In his critique, Kuo maintained that much of fungal taxonomy is unscientific, because classifications and keys often invoke morphological features that cannot be easily explained in terms of selective advantage (or, to put it in common terms, how those features may promote “survival of the fittest” for the individuals in which the features have evolved). The solution that he proposed is to base classifications on morphological features (a.k.a. morphological *characters* in the jargon of systematic biologists) for which a role for natural selection can be explicitly hypothesized, or even to discard the use of comparative morphology altogether, subjugating morphological features to the

role of identifying taxa that have been delimited using more “objective” methods such as DNA analysis and mating studies. The argument for explicitly including ecological hypotheses in mushroom classification is extremely compelling. It is, in fact, precisely because this alternative seems so reasonable that I feel compelled to provide arguments to the contrary, for there are a number of pitfalls in its use as the basis for a stable classification system. A second reason for my writing this article is also inspired by Kuo’s critique of the foundations of taxonomic mycology, as it raises a number of important issues about how taxonomy is practiced. I believe that an examination of these broader issues – for example, the goals, methods, and shortcomings of phylogenetic classification, and whether there can be a distinction made between unscientific and merely subjective classification – seem in order. My goal is not to produce a rebuttal to Dr. Kuo’s paper – I am in full agreement with the underlying problems that it identifies, and differ only in the remedies that I prescribe – but rather to draw upon its examples to examine mushroom classification in the light of natural selection, morphology, and molecular genetics. In this article, I will outline some of the pitfalls of eco-evolutionary hypotheses in the context of the primary goal of phylogenetic classification, examine the application (sometimes rigorous, often not) of comparative morphology in developing these classifications, and finally consider the questions of whether the current system of mushroom classification is fundamentally broken and whether DNA studies offer the solution to the problem of unstable classifications and the frequent name changes that follow from them.

Glossary

- **Genotype:** The genetic composition (i.e., DNA sequence) of an organism.
- **Homologous:** A character occurring in two or more species that was derived from the common evolutionary ancestor of those species (i.e., a character that does not occur due to convergent evolution).
- **Natural selection:** The process by which the best adapted individuals within a population of organisms survive and reproduce with higher frequency, passing their genes with higher frequency to the next generation. Most populations within a species contain a variety of genotypes and phenotypes. Because environmental constraints (e.g., food and other resource availability) generally limit the number of individuals that can survive in the population, those individuals having a trait that gives them an advantage in surviving long enough to successfully reproduce are the ones that pass on their heritable traits (those that can be passed from parent to offspring) more frequently to the next generation.
- **Phenotype:** the observable manifestation – e.g., biochemical, physiological, behavioral, ecological, or morphological – of the genotype, as modified by the interaction between the underlying genotype and the organism’s environment. Selective pressure refers to the intensity of natural selection; i.e., the level to which a particular environment favors particular characteristics of an individual. If we think of the environment – its climate and its ability to determine the availability of food, and its ability to determine mating success and the survival of offspring, for example – as a filter through which some phenotypes can pass (survive and reproduce) but others cannot, the degree of selective pressure would be analogous to the fineness of the mesh of the filter. High selective pressure means that some phenotypes are strongly favored over others; low selective pressure means that a wider variety of phenotypes will experience more-or-less equal survival and reproductive success.
- **Synapomorphy:** a shared, derived state of a homologous character (i.e., shared within the members of a taxon but in a state different from that occurring in the most closely related taxon from which it is distinguished).
- **Taxon** (plural: **taxa**): a named group of organisms at any level in a taxonomic classification (i.e., species, subgenus, genus, family, order, phylum, etc.).

SYSTEMATIC CLASSIFICATION AND THE ROLE OF EVOLUTIONARY HYPOTHESES

The primary goal of biological systematics is to build a classification system of organisms that

reflects their underlying *phylogenetic* relationships – those relationships that arise from genetic relatedness (i.e., shared evolutionary ancestry). As Hennig (1966) pointed out in his groundbreaking work on the topic, only this type of system can be linked reliably to (and unify the insights gained from) diverse areas of investigation such as morphology, genetics, behavior, ecology, and physiology. In addition, such a system allows the most power to predict the characteristics of species that have not been studied in detail, and is inherently the most stable in the face of additional data. For readers unfamiliar with phylogenetic methods, I recommend the introduction to the subject by Mueller and Pine in *McIlvainea* volume 11 (1994).

Though the goal of building a classification that reflects genetic relatedness may seem quite straightforward, getting there is often anything but. How do we go about assessing genetic relatedness? There is little doubt that the recognition of species and the grouping of species into more inclusive taxa (subgenera, genera, families, etc.) can be a highly subjective exercise. Classifications often appear to be (and sometimes are) based on little more than the ‘gut feelings’ of a taxonomist; furthermore, these feelings may be about seemingly insignificant differences in seemingly insignificant characters. Given that natural selection should favor some traits over others, it seems sensible that a classification of fungi will best represent evolutionary history by incorporating evolutionary process – in other words, by being based upon those characters for which a role for natural selection can reasonably be posited. This premise is the essence of Kuo’s argument that much of taxonomic mycology is unscientific, so I will summarize this argument below.

In illustrating his argument, Kuo presented the following hypothetical taxonomic key couplet:

1. Displaying a morphological feature whose predictive value.....Subgenus *Somethings*
is as far as we know coincidental, since we have no working
theory about how the feature is actually related to natural selection
and speciation.

1¹. Not displaying this feature..... 2

He then proceeded to contrast this couplet with the following one (I will hereafter refer to this as the “*Scleroderma* couplet”):

1. Rhizomorphs aggregated into a stemlike structure that holds.....*Scleroderma septentrionale*
the spore-producing machinery high enough to avoid being covered
with drifting sand on exposed beaches and dunes.

1¹. Rhizomorphs present but not aggregated into a stemlike structure..... 2
since the organism grows in wind-protected environments.

On its surface, the inclusion of this explicit eco-evolutionary hypothesis in the key is extremely sensible, as it places the distinction between characters in the context of a reasonable mechanism for how the process of natural selection may have proceeded – thus, the taxonomy of *Scleroderma* species appears to emerge from the realm of the subjective into that of the scientific. However, on further examination, the application of these hypotheses is problematic due to the presence of several pitfalls that I will describe below.

PITFALL #1: ECO-EVOLUTIONARY HYPOTHESES ARE AD HOC AND UNLIKELY TO IMPROVE THE SCIENTIFIC CONTENT OF THE CLASSIFICATION

In further outlining his argument, Kuo presented the following couplet derived from Hesler’s (1969) key to *Gymnopilus* subgenus *Gymnopilus*:

1. Spores 3.5–7 μm long; if 6–8 μm long, take next choice.....Section *Microspori*

1¹. Spores (6-) 7–9 μm long.....Section *Gymnopilus*

Of the classification represented by this couplet, Kuo wrote: "...it is self-evidently unscientific. Entire taxonomic divisions have been erected on the basis of whether the ring is ‘persistent’ or not, and on differences in spore lengths—without even a passing guess as to how these features might

relate to natural selection and evolutionary history in the genus." With that thought in mind, and following the example of the *Scleroderma* couplet above, I have revised the *Gymnopilus* couplet as follows:

1. Spores 3.5–7 μm long, the smaller size resulting inSection *Microspori*
increased dispersal potential; if 6–8 μm long, take next choice

1¹. Spores (6–) 7–9 μm long, resulting in increased longevitySection *Gymnopilus*
due to the additional nutrient reserves allowed by a larger spore volume

In the revised version, I have presented a plausible scenario as to how spore size might be related to natural selection and evolutionary history. Without a doubt, this story becomes significantly more stimulating with the addition of an ecological-evolutionary explanation, but the *scientific* content of the classification – that is, *the degree to which the available evidence corroborates the hypothesis that members of section Microspori share a more recent common ancestor with one another than with the members of section Gymnopilus*, and vice versa – is not improved in the least. That simply attaching a hypothesis to a couplet in a taxonomic key does not make the classification more scientific is a fact stated by Kuo; however, the degree of plausibility that can be attached to the *Scleroderma* couplet and the revised *Gymnopilus* couplets above are quite equivalent. Here, I will emphasize two central points. The first is that the type of hypothesis that is relevant to systematic classification is a hypothesis regarding the evolutionary relatedness of organisms; hypotheses pertaining to the actions of natural selection are at best irrelevant to – and may even run counter to – this goal. The second is that a scientific hypothesis is one that can be falsified by the collection of observational or experimental data – for example, the hypothesis of closer genetic relatedness (i.e., common ancestry) of a species within section *Microspori* to other species in *Microspori* than to species in section *Gymnopilus* can be rejected (or, alternatively, further supported) through the analysis of additional character data; whether the genetic relationships between *Scleroderma* species resulted from exposure to sand dunes falls more within the realm of conjecture. Hypotheses related to plausible mechanisms of natural selection can certainly provide interesting, and even testable, insights regarding the evolutionary process; however, their explanatory strength is a matter separate from the phylogenetic classification and must be evaluated once the classification is in place (e.g., by measuring how consistently differences in traits are correlated with specific environmental differences, assessed in relation to the phylogenetic classification). The inclusion of such hypotheses in building a classification simply superimposes ad hoc speculation upon the underlying problem – distinguishing taxa on the basis of an observed difference in a single character – rather than offering a solution to this problem.

PITFALL #2: NOT ALL EVOLUTION PROCEEDS VIA SELECTION ON ECOLOGICAL TRAITS

If all evolution proceeded via natural selection for ecological traits of the basidiome that were easily observable, then the application of mechanistic hypotheses would be sensible, at least in cases where none of the other pitfalls that I describe below apply. However, evidence suggests that this is not the case. Though ecological speciation (divergence of populations under different selective pressures imposed by different environments) appears to be a common mode of speciation (Schluter, 2009), there is also ample evidence that “mutation-order speciation” occurs, where random genetic mutations arise and populations diverge into separate species even when facing similar environments (Mani & Clarke, 1990; Schluter, 2009).

In studies of mushroom-forming fungi, probably the most commonly-collected ecological data are observations of associations with plant species. When plant host associations have been carefully documented, specialization of species on particular hosts sometimes occurs, as in the example of *Pleurotus populinus* in North America (however, *P. ostreatus* and *P. pulmonarius* are not distinguishable by host; Vilgalys et al., 1993). In some other examples, however (e.g., North American alpine and subalpine *Laccaria*; Osmundson et al., 2005), there is no discernable correlation between genetic species and their plant associations. Basing classifications on host data is certainly comprehensible in terms of natural selection and can be quite useful for distinguishing species in some cases, but will be misleading in others.

Data on ecological communities in general provide a problematic foundation for phylogenetic classification. As a rationale for this point, we should consider that a given species (1) did not necessarily evolve within the ecological community where it is now found; (2) quite likely did not evolve under the environmental conditions in which we now find it; and (3) did not necessarily evolve either in response to or in parallel with the other species with which we now find it. Any of the above conditions – for example, the likely (and probably quite common) scenario in which closely related populations of a species became isolated at some point during evolutionary time (by environment or by physical barriers such as the formation of mountain ranges, oceans, etc.), developed biological barriers that prevented mating between the isolated populations, and then were brought back together by geology, long-distance dispersal, or human introduction – would confound these kind of ecological inferences.

PITFALL #3: SELECTION MAY OCCUR ON “HIDDEN” ECOLOGICAL TRAITS

Even when ecological speciation occurs, recognizing characters of selective importance may be a difficult, in addition to a subjective, exercise. The tendency to make assumptions regarding selection for traits of the basidiome ignores the possibility that the characters under the strongest selective pressure may instead be biochemical, physiological, or otherwise difficult to observe. For example, the ability of ectomycorrhizal (EM) fungi to use nitrate as a nitrogen source (as opposed to ammonia, the more commonly-used source) is ecologically and evolutionarily relevant, with importance for mycorrhizal associations, nutrient cycles, and the ability of species to respond to increased human-mediated addition of nitrate to soils via fertilization and air pollution (Nygren et al. 2008). Different groups of EM fungi appear to respond differently to nitrate, and this trait is likely to have an evolutionary basis. Another example comes from the study of Agerer (1999), who examined the anatomy of rhizomorphs in the *Boletales* and found these anatomical features to be highly consistent with underlying genetic relationships – more so, in fact, than most basidiome characteristics. One could quite readily formulate explicit hypotheses regarding natural selection in both of these examples, as both nitrate metabolism and rhizomorph morphology are likely to play key roles in nutrient acquisition, and therefore play a key role in defining the ecological niche of fungal species. Both examples also describe traits that are likely to be missed in an examination of basidiome morphology.

PITFALL #4: ECOLOGICAL TRAITS OFTEN EXHIBIT CONVERGENCE

The evolution of vertebrate forelimbs modified as wings almost certainly has a strong relationship to natural selection, as having wings could allow increased access to insect prey and increased opportunity for escape from terrestrial predators. Using the character of wings as a sole basis for classification would lead to placing birds and bats as close relatives. Through the careful examination of the structural details of both groups of organisms, however, comparative morphologists have long known that wings in birds and bats did not arise due to common ancestry but evolved separately, an example of what is known as *convergent evolution*.

In cases where species arise from evolutionary radiation, i.e., the specialization of different populations on distinct ecological niches (examples include Hawaiian Silversword plants, African rift lake cichlid fish, and the Darwin's finches of the Galapagos Islands), then the kinds of characters under ecological selection may correspond to phylogenetic grouping criteria. However, a major ecosystem characteristic, such as frequent prolonged drought, would be expected to place similar selective pressures on many genetic lineages simultaneously; in these cases, we would expect to observe convergence in characters due to shared environment, not genetic relatedness.

The sequestrate habit in mushrooms has widely been hypothesized to represent an evolutionary adaptation to prevent desiccation of the spore-producing surfaces (Kendrick, 1994b). The genera *Cystangium*, *Gymnomyces* and *Arcangeliiella*, despite having several microscopic features similar to those found in the agaricoid genera *Russula* and *Lactarius*, were formerly placed in the *Hymenogastreales* along with other sequestrate fungi. Examination of additional features, including DNA sequences, has led to the placement of these fungi within the *Russulales* (e.g., S.L. Miller et al. 2001; Kendrick 1994a). Examination of other sequestrate genera has similarly led to their

classification alongside non-sequestrate relatives (Hibbett et al. 1997; Peintner et al. 2001; Kendrick 1994b). Here, the character of supposed ecological (or selective) relevance exhibits convergent evolution, whereas other characters, such as amyloid basidiospore ornamentation and sphaerocysts in the case of the russuloid sequestrate genera, reliably track the underlying genetic relationships, even though their relation to natural selection would be more difficult to explain. A second example of convergent evolution in mushroom-forming fungi is the ability to form ectomycorrhizal symbioses. Ectomycorrhizal mushroom genera are scattered across both the ascomycetes and basidiomycetes, rather than found to be closely related to one another (Hibbett et al., 2000). Thus, the ability to form ectomycorrhizae is not a good character upon which to build classifications when used in isolation (however, it can be quite useful in combination with other characters, such as in the separation of *Tapinella* from *Paxillus*; Sutara 1992), even though it is undoubtedly one of the most critical events to occur in the evolution of fungi and almost certainly a character strongly related to natural selection. These examples show that building classifications based on hypotheses related to major ecological characteristics may well lead to incorrect conclusions due to the occurrence of convergent evolution, in cases where inferences drawn from comparative morphology may correctly reveal the underlying phylogeny. They also point out an important pitfall with the comparative morphology approach, along with its antidote. After all, the traditional concept of the Hymenogastrales was based on morphological considerations, not ecological ones: the placement of the russuloid sequestrate fungi in the *Hymenogastrales* rather than in the *Russulales* resulted from decisions regarding the *weighting* of characters; i.e., the decision by a taxonomist about which characters best represent the underlying genetic relationships. The solution to the problem of subjectivity in weighting is the use of corroborating evidence as an alternative to relying on single-character taxonomy (see “On the use – and misuse – of comparative morphology in building classification systems,” below).

To reiterate an important point, the goal of a systematic classification is to represent *genetic* relationships, not ecological ones. When used in a rigorous manner, with the totality of available characters (behavioral and ecological as well as morphological and molecular characters can be applied), the pattern of synapomorphies (evolved characters that are shared within a group; i.e., “defining characters”) are more likely than natural selection hypotheses to reflect the underlying phylogeny. As pointed out by Hennig (1966), “It is not the relation between the species and the space and its living conditions that are in themselves of primary interest from the systematic standpoint, but the [genetic] inter-relations between the individuals that are distributed throughout the space.” A classification that is based on an ecological trait does not necessarily agree with – and is even likely to conflict with – a phylogenetic system.



[back to top](#)

PITFALL #5: SELECTION ON WHICH ECOLOGICAL TRAIT?

Although when we think about classifications it is most often the relationships between species that interest us, a classification must also account for relationships even between different life stages of a single individual if it is to represent genetic relatedness. For instance, a classification should certainly group together the tadpole stage and the adult stage of a single frog species – a system in which the tadpole stages of two different frog species are grouped more closely to each other than they are to their respective adult stages would fail to capture underlying genetic reality and, in doing so, misinterpret the evolutionary history of the organisms. Building a classification on supposed ecological adaptations can unfortunately reach much the same result. Consider the following example provided by Hennig (1966):

The significance for biological systematics that attaches to the variability of the individual in time is that, strictly speaking, one and the same individual assumes a different place in most ecological systems at different times of its life. At first sight this fact seems peculiar, but immediately becomes evident if it is clarified by a very simple example. The larva of the May beetle assumes an entirely different place in an ecological system, that is, in a system that seeks to present the whole of all living organisms as a community, than the sexually mature beetle does. In this system the

larva would be more closely associated with other animals that live in the ground and eat roots than with the imago of the May beetle into which it later develops. The imago would be more closely associated with other (flying and leaf-eating) animals. The same applies in countless similar cases for most of the imaginable morphological and physiological systems.

As Hennig pointed out, a single individual organism may have very different ecologies at different life stages of its life cycle. The larva of a May beetle and the adult beetle possess a very different set of traits, and exist in different habitats that almost undoubtedly exert very different types and levels of natural selection.

Because many fungi have multiple life stages, this is an important point for mycological classification as well. The sporocarp (“mushroom”) represents only a single stage of the life cycle of the fungal individual; i.e., meiosis and the production of sexual reproductive propagules (meiospores) – the sexual reproductive phase of the life cycle is referred to by mycologists as the **teleomorph**. Many fungi (especially in the *Ascomycota*) also have an asexual sporulating phase (known as an **anamorph**), and many fungi persist only as mycelium for long periods of time, assimilating nutrients, prior to forming sexual sporocarps. Both the anamorphic and mycelial phases may be extremely important in the growth, development, and spread of the individual; therefore, there is no reason to assume *a priori* that natural selection works preferentially on teleomorph (sporocarp) characters rather than on characters of the mycelium or, where present, the anamorphic stage. An excellent example of the importance of anamorph characters for phylogenetic reconstruction is provided by the work of Saenz (1998; Saenz & Taylor 1999) on the evolutionary classification of the powdery mildews (*Ascomycota*, *Erisyphales*). The powdery mildews produce sexual reproductive structures (ascoma) that often have distinctive appendages; these appendages, as well as the number of asci per ascoma, have traditionally been used to distinguish genera. In analyzing both DNA sequences and 17 morphological characters, Saenz found that morphological characters of the anamorph, rather than of the teleomorph, were in general a better indicator of the underlying genetic relationships.

It must also be emphasized that selective pressure can exert itself at the level of individual traits. An individual organism then, even within a single life stage, is an “evolutionary mosaic,” where different traits respond differently (some not at all) to the same set of selective pressures (i.e., to the same environment). How do we know that natural selection is working most significantly on the stipe (as in the *Scleroderma* couplet), and not on nitrate assimilation, rhizomorph morphology, or some other trait? To be sure, it is possible that the *Scleroderma* couplet contains a reasonable hypothesis – that the stipelike aggregation of rhizomorphs in *S. septentrionale* is the product of selective pressures related to exposed beach and dune habitats. *Hebeloma cylindrosporum*, *Laccaria trullisata*, and *L. maritima*, to name just a few examples, also appear to be specialized on exposed dune habitats and exposed sandy soils (Gryta et al., 1997; Mueller, 1992), and all have a well-developed stem to raise the spore-producing tissues away from the sand-covered ground surface; however, the vast majority of the other species in these genera have the very same morphological structure but live in wind-protected habitats. Other examples of ecological hypotheses illustrated by Kuo – “Perhaps the scales on the cap of one species represent an adaptation to drier ecosystems, handily holding precious moisture on the mushroom rather than letting it slide away. Or perhaps the same scales are the side-effect of an adaptation for a thicker, denser cap surface that protects the vital spore-producing hymenium below it from increased sunlight. Perhaps a species has developed darker pigments to hold heat in low-sunlight forests” – are likewise possible; however, it is also possible that these features are *selectively neutral*; i.e., having them, or not, does not affect the survival and reproductive success of an individual in a given environment. The observation that scaly- and non-scaly-capped species, or light- and dark-colored ones, often occur in the same habitat – often on the same log, for example – suggests that selective neutrality for such characters is a strong possibility. Building a systematic classification upon selective scenarios for these traits would be a highly subjective exercise.

CHARACTERS OF UNKNOWN OR DUBIOUS SELECTIVE ADVANTAGE MAY BE CONSISTENT WITH THE UNDERLYING PHYLOGENY

When I consider mushroom classifications developed prior to the use of DNA in systematics, I am often surprised not as much by their shortcomings (of which there are certainly plenty) as by the number of times in which they actually appear to be correct when confronted by additional data. Singer (1986) didn't need DNA evidence to provide the link between poroid boletes and the lamellate genera *Phylloporus*, *Hygrophoropsis*, and *Paxillus*, for example. And sometimes, the oddest of distinguishing morphological characters are supported by molecular data. Take siderophilous granulation, as just one example. A process that resembles alchemy more than science, a test for siderophilous granulation involves heating a gill fragment mounted in acetocarmine on a glass slide, stirring with a rusty nail, repeating this exercise 2 or 3 times, then observing under a microscope for the presence of dark purple- to black-staining granules in the basidia (Lange & Hora, 1963; McAdam, 2007)...“Eye of newt, and toe of frog, wool of bat, and tongue of dog...” (Shakespeare, *Macbeth*). I think that I speak for many others who have employed this rather bizarre procedure in saying that I would not be the least bit disappointed to find that it were *not* a taxonomically useful exercise and could therefore be discarded. To our great chagrin, however, DNA evidence supports siderophilous granulation as a useful taxonomic character in the *Lyophylleae* (Hofstetter et al., 2002). Another prominent example of a morphological character of extreme taxonomic importance is the presence of asci versus basidia for meiospore dispersal. Are there strong, ecologically-relevant differences in dispersal ability between the two meiocyte types? Are there discernable differences in the habitats in which ascomycetes and basidiomycetes grow? Though more detailed ecological data may provide some surprising answers, it seems quite possible, based on the observation that ascomycetes and basidiomycetes are often found in the same habitats and play many of the same ecological roles, that the answer to both of these questions is no. Though it is difficult to concoct a hypothesis surrounding the selective relevance of either siderophilous granulation or meiocyte type, both appear to be strongly consistent with the underlying phylogenetic relationships that are indicated by DNA analyses. In other words, odd morphological characters often *work* for grouping organisms by genetic relatedness. As our objective should be to identify those characters that represent the underlying genetic relationships between individuals, species, and higher taxa, the question of whether or not these characters lend themselves to the formulation of natural selection hypotheses is largely irrelevant. So, am I arguing that habitat and other ecological information should be ignored by mushroom collectors? Absolutely not. What we learn about a fungus, and the informational value of our collections for ecology, conservation and management – and sometimes systematics – rises significantly with the inclusion of such data. I will return to this point later in my conclusions.



[back to top](#)

ON THE USE – AND MISUSE – OF COMPARATIVE MORPHOLOGY IN BUILDING CLASSIFICATION SYSTEMS

So, if morphological characters have the potential to (even though they cannot always) adequately represent the phylogenetic relationships between organisms, then why are so many ‘traditional’ classifications being rearranged due to DNA analyses? In addition, many of the criticisms that I have made about eco-evolutionary hypotheses apply equally well to the use of a character such as spore length for distinguishing taxonomic groups – spore length, like pseudostipes, scaly protective cap coverings, and dark pigments, may undergo convergent evolution, be parts of evolutionary mosaics, and be selectively neutral – can the use of comparative morphology be justified given these issues? I believe that the answer to both of these questions can be found at least partly in the way in which character data are used to formulate classifications; in other words, the problem is often not in how the associations of characters are *hypothesized*, but rather in how they are *analyzed*. In some cases, these analyses have been conducted rigorously; in others they are nearly or completely lacking.

Systematics (the science of biological classification) is a science of corroboration, where available

data are considered in their totality in order to discern the pattern of genetic relationships between taxa. In its most rigorous form, derived from the ideas of Hennig (1966), the characters are analyzed and the classification is based on those characters that are *synapomorphic*, or shared, derived characters – i.e., shared by a group of species, and existing in a changed state relative to the nearest phylogenetic neighbor and the common ancestor of the two groups. Classification based on synapomorphic characters (a *phylogenetic* classification) can be contrasted with classification based on overall similarity of features (a *phenetic* classification), the latter of which is subject to the same types of pitfalls that I have described for eco-evolutionary hypotheses, even though the two methods arrive at the same classification in some cases. Comparative morphology is a method for determining the patterns of genetic relatedness between organisms through the determination and analysis of **homologous** characters (characters derived through common ancestry) to reveal patterns of synapomorphy. Its cornerstone is the corroboration of data. Although the name 'comparative morphology' suggests that only morphology is used, other forms of data (e.g., molecular, chemical, physiological, ecological, and behavioral) are perfectly compatible with its methods and objectives, which form the cornerstone of biological systematics as a whole. Far from being atheoretical, comparative morphology involves the development of specific hypotheses and tests them using multiple lines of evidence. There is a hypothesis implicit whenever a taxonomist uses a specific character (or characters) to distinguish taxa: that a form of the specified character is shared within the members of a taxon but not the taxon from which it is distinguished; i.e., that it is a synapomorphy for that taxon. This hypothesis can in fact be subdivided into several parts: (i) the hypothesized homologies of the characters selected for analysis; (ii) the hypothesis that taxa sharing a certain character state – that is, the specific form that a character takes in a taxon (e.g., for the character of spore length, a state for that character could be 'longer than 10 microns') are each others' closest genetic relatives; (3) the classification itself – which may or may not be depicted in the form of a phylogenetic tree – presents a hypothesis of relationships that is open to further testing using additional data and/or taxa.

It is therefore not the lack of a theoretical basis that is in question for comparative morphology, but the degree of support that data provide to a given hypothesis. Comparative morphology and systematics are not unscientific. However, the sound theoretical basis of the science does not by any means guarantee the quality of the hypotheses generated by its practitioners. In many cases researchers have generated systematic hypotheses in a subjective manner (i.e., the 'gut feeling' of a taxonomist), without rigorously using additional data to corroborate or refute these hypotheses. Such classifications – even when formulated by taxonomic experts – are weak at their foundations; it should come as no surprise to see them overturned by the examination of molecular or other additional data, given the lack of rigor with which they were formulated. A systematist that selects single characters arbitrarily (with this process sometimes going under the name of "expert opinion") and builds classifications upon them is engaging in poor systematic practice, whether a hypothesis about natural selection can easily be attached to the character or not. The rigorous application of systematic theory takes all of the available known characters under consideration, and seeks to determine underlying patterns of genetic relationship through the analysis of synapomorphic characters. This method stands in contrast to that of building classifications through the use of eco-evolutionary hypotheses, which simply attaches a nice story to a single-character approach to classification that ignores the importance of homology assessment.

Although rigorous phylogenetic analyses are commonly used on molecular data, their use on morphological data is much less common for fungi; however, an excellent example to the contrary is the phylogenetic analysis of morphological characters in Mueller's (1992) monograph of North American *Laccaria*. Several reasons exist for the relative lack of rigor in treating morphological data in fungi. One is that the methods used for phylogenetic analysis are computationally demanding when more than a handful of taxa are included. The advent of DNA sequencing technology parallels quite well the widespread availability of the computing power sufficient for such analyses, and the large number of characters analyzed from DNA data makes the use of automation a necessity, so it is quite understandable that DNA data are often more rigorously analyzed than morphological data – many of the classic monographs for fungi were written before Hennig's work (and therefore lack the theoretical grounding provided by Hennig) and/or before the availability of the computational resources necessary for large-scale analyses. Another reason is that, compared to most animals and plants, fungi are morphologically simple, resulting in a paucity

of characters available for analyses. And another reason, I suspect, is sociological; for some reason, mycologists just didn't take up these methods to the extent that their botanist and zoologist colleagues did. Even in cases where such explicit analyses were not performed, however, traditional classifications have often been built on the basis of corroborating data; for example, the well-known classification of Singer (1986) considered numerous characters in its construction. It would be unfair to portray such studies as atheoretical, though the lack of explicit character analysis allows subjective decisions on the emphasis of different characters (i.e., character weighting) to become a significant issue.

In his criticism of comparative morphology, Kuo suggested that the process as advocated by Korf (2005) is nearly equivalent to "if two things look different to me, they must be different." As I interpret Korf's article, however, the author does not advocate such a subjective approach to classification; in his criticism of single-gene "phylogenies" and systematic classifications based on too few genes and/or taxa, and his advocacy of the phylogenetic methods of Hennig, Korf defended a scientific approach based upon corroboration using multiple lines of evidence. And though Korf's 'curmudgeonly' argument has been widely interpreted as a push against the use of DNA sequence data in phylogenetics, I believe that this is not the case. Rather, Korf's article is critical of two specific aspects of the application of DNA data: (i) the definition of species by a specific, single DNA sequence, termed a "DNA barcode," and (ii) the preferential funding and scientific respect given to DNA-based studies that do not result in the collection of new specimens. The first of these aspects is an extremely valid criticism – a taxonomy based on DNA barcodes is a single-character taxonomy, indistinguishable from a single-character morphological taxonomy except in its technological complexity, and several genetic phenomena (e.g., horizontal transfer of genes between species, variation in the rate of evolution between genes, and variation in the rate of lineage sorting, the process by which individual gene histories converge to that of the overall species phylogeny) occur that often preclude the accurate estimation of species phylogenies from analyses of single genes. The second has some merit as well: although I am thoroughly convinced that the re-examination of existing collections and classifications using DNA data is both important and useful (not to mention that a vast number of existing herbarium collections have never been thoroughly examined, using any criterion, in the first place), our planet's biodiversity crisis demands that field surveys should be accorded high priority in science's intellectual and funding agendas.

IS DNA THE ANSWER?

Molecular characters have largely supplanted morphological ones in the determination of systematic classifications for the fungi. There are both advantages and disadvantages to this approach; therefore, I think that it is worthwhile to take a look at both molecular and morphological data in terms of their roles in evolutionary inference and their relation to hypotheses about natural selection, and to examine whether molecular data are inherently more scientifically rigorous and more likely to produce stable classifications than are morphological data.

The greatest difference between molecular and morphological data is that molecular data (specifically DNA, the genetic informational molecule of the cell) represent the organism's *genotype*, and morphological data represent its *phenotype*. This distinction is a critical one in comparing the two approaches. Given that the objective of a systematic classification is to reflect genetic relatedness, it is of great advantage to examine the genotype directly – the analysis of DNA sequence data is therefore an extremely powerful tool for inferring evolutionary patterns. In addition to representing a more direct way of assessing genetic relatedness, molecular data may (though do not always) lend themselves more easily to homology assessment, and may contribute a larger number of analyzable characters (a typical DNA region used in phylogenetic analyses consists of hundreds or thousands of individual pieces called nucleotides) compared to morphological data. DNA is, biochemically-speaking, a relatively simple molecule consisting of a limited number of chemical components (4 major nucleotide types in DNA), and molecular biology has shed significant light on the mechanisms of mutation and on the patterns of DNA sequence divergence between species. This mechanistic comprehensibility should not, however, be conflated with the possession of a clear link to the process of natural selection. That is because natural selection occurs at the level of the *phenotype*, not the genotype. While change at the DNA level is necessary for trait changes in an evolutionarily significant way (because evolution by natural

selection requires heritability), natural selection does not “care” whether there is a cytosine or an adenine nucleotide at position 123 in gene X (i.e., the *genotype* of the organism); instead, it acts upon the differences in a trait or traits that may result from this change (i.e., on the *phenotype*). If there is no selective advantage (i.e., advantage in survival and reproduction) in one genotype relative to the other, then it is a mistake to assume a causal link between natural selection and the pattern of genetic relatedness drawn from these DNA differences.

Drawing direct causal links between molecular evolution and natural selection is problematic for at least two reasons. One reason for this disjunction is that much, even most, molecular evolution is either selectively neutral or actively selected against. In parts of the genome that have direct functional roles such as encoding proteins or RNA, genes generally experience functional constraint – in essence, the pressure to not evolve. The majority of retained mutations (and thus observable via DNA sequencing) in DNA sequences occur in stretches of the genome that do not encode proteins, or in the third codon positions of protein coding genes, where changes in the DNA sequence generally do not lead to changes in the amino acid sequence of the resulting protein (called silent, or synonymous nucleotide substitutions). Because mutations accumulate slowly over evolutionary time, the comparison of closely related species must usually be made using DNA regions that are under low functional constraint; i.e., regions containing mostly neutral mutations. While these regions can be extremely useful in establishing genetic relationships, their association with natural selection is questionable – in fact, it could be said that natural selection has ignored them.

A second reason is that complex morphological and ecological characters do not necessarily show a one-to-one correspondence with gene sequences; for example, complex traits may occur from the interactions of several or many genes, and regulation of a gene (when it is turned “on” or “off”) may be just as important as its DNA sequence in determining traits. Furthermore, a single gene can serve multiple roles, interacting with different genes, in different pathways, to produce different products. The search for symbiosis-related “master genes” in ectomycorrhizal fungi is a potent example. While the recently-sequenced *Laccaria bicolor* genome does contain a number of genetic innovations, with at least some of these appearing to be involved in the formation of symbiotic tissues, research on gene expression profiles in symbiotic and non-symbiotic tissues indicates that many genes are either upregulated (i.e., their expression is “turned up”) or downregulated (expression is “turned down”) during mycorrhizal formation but that these genes exist in non-mycorrhizal as well as mycorrhizal species (Morel et al., 2005; Martin et al., 2008). In other words, it appears quite likely that many aspects of ectomycorrhizal symbiosis involve a different use of genes that already serve other purposes in the organism. In such cases, linking molecular differences to natural selection is difficult, if not impossible.

In summary, although molecular data are useful for uncovering phylogenetic relationships, they are arguably worse than morphological data in terms of reflecting the action of natural selection on species. However, molecular data can be instrumental in examining morphological evolution by evaluating classifications based on competing concepts of character weighting and, in cases where few morphological characters are observable or where extensive convergent evolution has taken place, molecular data may be our only hope of uncovering the underlying phylogeny. Are they infallible for producing accurate systematic classifications and illuminating patterns of morphological character evolution? Probably not. We are far from a truly mechanistic understanding of how evolution proceeds at the molecular level in relation to natural selection. The interaction of multiple genes and their influence on traits, the mechanisms of gene regulation, the effects of alternative splicing of RNA transcripts (allowing the formation of multiple, distinct products from a single gene), and the action and function of small RNAs are just a few ways in which the biochemical and ecological functions of organisms fail to be adequately represented by simple comparisons of DNA sequences. In addition, comparisons of phylogenetic trees obtained from one or several genes with those constructed from genome-level comparisons suggest that the number of genes commonly used in molecular phylogenetic studies – even the massive Assembling the Fungal Tree of Life project – is far too low (Rokas et al., 2003), and that the genes commonly used in fungal phylogenetics perform poorly relative to other genes for obtaining accurate phylogenies (Aguileta et al., 2008). Therefore, while DNA is certainly a powerful tool for establishing the genetic relationships between organisms, and will only become more so as a larger number of complete genome sequences are obtained, it is much too early to conclude that

recent classifications based on molecular data are likely to be stable.



[back to top](#)

CONCLUSIONS

It is doubtlessly the case that mushroom classification is currently undergoing a shift of historic proportions. Although the study of microscopic characters led to taxonomic rearrangements such as the joining of divergent sporocarp morphologies (e.g., gilled, poroid and sequestrate forms) within taxa, molecular data have led to a much more extensive overhaul of the Friesian classification system. Is this large-scale destabilization a necessary consequence of a broken system finally getting a badly needed fix, or simply the progression of science aided by new types of data? Probably a little of both. Subjectivity in character selection and weighting is certainly a significant part of the problem; in many cases, such problems could probably have been avoided by the rigorous application of comparative morphology. In other cases, however, morphological characters – even rigorously analyzed – would not have prepared us for what DNA is saying about the evolutionary relationships between fungal species. Morphological characters of the sporocarp may be too few, may have experienced too much convergent evolution, or may be secondary to “hidden” characters (mycelial, anamorphic, etc.), thus obscuring evolutionary patterns; herein is the benefit of looking directly at the genotype in order to ascertain evolutionary relationships. Although one might argue that we should therefore abandon comparative morphology as a method for building systematic classifications, I would argue against such a practice, arguing instead for a role for both morphological and molecular studies. One reason for this position is that we are often primarily interested in the phenotype of a mushroom – is it poisonous? A choice edible? Does it produce pigments useful for fabric dyeing? Does it have useful medicinal or chemical compounds? A second reason is that the mushrooms that we wish to identify are, first and foremost, morphological entities. The degree of relationship between *Amanita phalloides* and *Amanita caesarea* would be of little interest to most mycophiles if removed from our morphological (and culinary, and toxicological) concepts of these two species. Indeed, the very names of species draw upon a morphological concept, and even the most “grind and go” approach to DNA analysis of herbarium collections is therefore indebted to morphological taxonomy for defining the entities to be studied (see Bunyard 2009 for a discussion of the intertwining of field and laboratory systematics). Incorporating morphological characters into systematic analyses allows their consistency with phylogenetic patterns to be rigorously evaluated. However, where inconsistencies between phylogeny and major morphological characters occur, frustration with the phylogenetic classification may ensue. In a letter to the editor of *Fungi* magazine, Leonard (2008) argues against the universal application of an evolutionary classification of mushrooms on the grounds of practicality, arguing that such a system does not work for the purpose of identifying fungi in the field, in poisoning cases, or in other situations where a morphological identification is the primary goal. While the familiarity of the Friesian system and its reliance on major morphological characters can certainly make placement of a specimen into a genus or family easier, I am not convinced that there is a clear dichotomy between having an evolutionary classification on the one hand, and being able to identify specimens on the other. Reconciling the two is in many cases a matter of perception. In some of these cases, evolutionary classifications reveal morphological character differences that had been previously overlooked or deemphasized. An example is the genus *Psilocybe*, where DNA studies show the blue-staining, psilocybin-containing species to be evolutionarily distinct from the non-bluing species. In such a case, separating the two groups on a foray identification table would not create additional difficulty for the participants. In other cases, such as when a new classification combines species having different spore print colors, perhaps it is worth asking why we emphasize the character in question to the extent that we do. I suspect that, often, the reason is as much historical as operational; we are simply used to classifying mushrooms using this character. However, a character such as the color of the spore deposit is just one character, and should be considered in the context of the totality of the characters before us; in other words, we may need to change our perception to one of looking at several characters simultaneously rather than just one at a time.

I think that we also need to ask whether an artificial classification system really does work, or what it works for. Even if it allows easier classification of an unknown specimen, the resulting classification doesn't really tell us anything. In the cases of identifying specimens implicated in a poisoning case, for example, our goal is to determine whether the specimen at hand belongs to a toxic group. In most cases, we are interested in characteristics at the species level – for example, we want to know if the species is *Amanita phalloides*. This identification follows from its sum of characters – olive pileus, free, white lamellae, white spore print, membranous annulus, saccate volva – regardless of what genus or family it is currently placed in. If our species is of unknown toxicity (or another characteristic of interest), then an evolutionary classification is more likely to provide a reliable answer than is a classification based on overall morphological similarity. The value of placing a specimen in an artificial classification, in contrast, is unclear, as the classification to which such a name belongs lacks any meaning in terms of the genetic relatedness of its members to one another. Assessing relatedness is not just an academic exercise; it has meaning for understanding the biology and properties of unknown species. If facilitating the rapid assignment of collections to larger groups is the primary goal of a classification, then we would be well-served to go back to classifying all gilled mushrooms as *Agaricus* and most tubulose-hymenophored fleshy mushrooms as *Boletus*; however, I suspect that this is not truly our goal. In other words, we know that there is information content to be gained from a finer-grained approach to classification. Certainly, there are cases in the DNA age where a finer-grained approach may simply tell us that two species are more related genetically without shedding any light on morphological identifying characters (for example, the insight that given morphologies such as truffle-like forms have evolved in numerous groups of fungi is in itself interesting, but not necessarily helpful at the identification table). However, adhering to the “traditional” classification shackles us to historical concepts of species relatedness; it forces us to honor the primacy of certain characters rather than encouraging us to view these characters of a mushroom in their totality, as part of a suite of features. Should we cling doggedly to Fries' ideas – based on the data available to him in the 19th century – on which characters to emphasize? I suspect that, given the additional data that have accumulated since his work, even Fries himself would not.

The analysis of DNA sequence data will continue to illuminate our understanding of evolutionary relationships in fungi, and the accuracy of its findings will most likely improve as additional data are gathered. In the meantime, however, it is providing some answers that many mycophiles may not want to hear – in some cases (e.g., *Strophariaceae*, *Boletales*), there will most likely be an explosion in the number of genera, while in other cases genera that appeared to be distinct will be subsumed into other, now more variable, genera (e.g., the absorption of *Dictyophora* species into *Phallus*). In evaluating the results of DNA studies, it is important to keep in mind that DNA is simply another type of data, subject to the same errors of classification based on overall similarity, subjective character differences, etc. as any other kind of data. Its use is no more scientific than that of morphology, and in both cases, rigor of the analysis is paramount – DNA sequences, like morphological characters, can easily be subjectively or sloppily analyzed.

As more data are collected for more taxa, and as new taxa are discovered, classifications are likely to become more stable. However, the goal of stability itself is of no value to science if maintaining an existing classification or an existing Latin name contradicts the current state of understanding of the evolutionary relationships between species. In quoting a passage from A.H. Smith's *Mushrooms in Their Natural Habitats*, Kuo attributes to Smith an understanding that “taxonomy is perpetually unstable because it is an elaborate hierarchy of hypotheses (read: ‘potential house of cards’) that may prove to be untenable.” I would like to offer a slightly different interpretation, embodied in the following quotation by E.F. Luttrell (1958):

Stability in the system of classification is impossible. Criticisms directed against essential revisions, therefore, are without basis. No such limitation is placed on any other science. What physiologist, for example, would accept as valid criticism a complaint that his work was making inadequate the knowledge acquired in the classroom ten, twenty, or thirty [or 170+ in the case of Friesian classification] years ago?

Change reflects the progress of the science, but only when it emerges through the rigorous

consideration of adequate amounts of data that are appropriate to the questions being asked. Researchers involved in changing classifications should therefore consider the burden of proof in doing so to be heavy, and the consequences of sloppiness great. And, in the end, we may never reach the answers to some questions about evolutionary relationships, as the specter of extinction hovers perpetually over the science of evolutionary inference – the morphological or molecular missing link between groups of organisms may be long extinct, obscuring the evolutionary patterns between contemporary organisms.

Classifications that are based on single character differences often have a foundation in subjective decisions about the weighting of characters; as a result, they are likely to be unstable and should be viewed (and made) with caution. The use of characters having some assumed selective advantage not only fails to correct this instability, but is likely to lead to incorrect hypotheses of relationship – such a practice has understandable motivations, but is problematic in its results. The most promising path to achieving a classification system that reflects underlying evolutionary relationships is to collect and analyze as many data – morphological, ecological, physiological, and genetic – for as many species as possible. We must also recognize that, even then, our classifications are likely to require change as new data sources, such as improved visualization tools for cellular and subcellular processes, the increased availability of whole-genome DNA sequences, and the improved characterization of gene regulatory processes, become available. None of the preceding critique should be taken to mean that we should not build hypotheses regarding ecological speciation and test them; quite the contrary – I am quite sure that it is here that many of the fascinating details of the evolutionary process await discovery. We must simply keep in mind that testing mechanistic hypotheses is a very different exercise than that of building evolutionary classifications – one that can only be reasonably done after the underlying genetic relationships are understood. Such analyses must follow the construction of a robust phylogeny, not the other way around.

Kuo's suggestions for improving the scientific value of field collections are right on the money, and I hope that they will become standard practice among collectors at all levels of expertise. He is absolutely correct in pointing out the lack of ecological data collected by most mushroom collectors (professional and amateur), and in calling for collectors to collect accurate ecological data with their field collections. The scientific and environmental benefits of such a practice are numerous – it can help us to better discriminate similar-looking species, predict when and where to find particular species, understand the adaptation of species to particular habitats, understand the complex ecological functions in which fungi play integral roles, characterize the rarity, habitat requirements, and level of threat to species, and develop plans and tools for conserving fungal diversity.

While the advancement of fungal systematics will benefit greatly from the expansion of molecular databases to include as many gene sequences for as many species as possible, it is critical for researchers and funding agencies to recognize that many of the missing pieces in the evolutionary puzzle remain waiting in the field for discovery. Historical extinction is among the greatest barriers to reconstructing evolutionary relationships (convergence in morphological and molecular traits is another), but as-yet-undiscovered contemporary taxa also contain missing data that hamper our efforts to build classifications out of the remaining pieces. Critical pieces of the fungal taxonomic and ecological puzzles, such as the discovery of the bizarre ascomycete genus *Pseudotulostoma* (O.K. Miller et al., 2001) and determination of an ectomycorrhizal habit for pleurotoid *Russulaceae* that can grow well off the ground on the trunks of standing trees (Henkel et al., 2000), have been obtained through field surveys of undercollected regions, particularly in the tropics. Modern-day extinctions pose a credible threat to reconstructing the fungal evolutionary tree, and keys to understanding specific ecological innovations or taxonomic relationships may be lost before we discover them. The calls to action made by Korf, Kuo, Bunyard and others bear echoing here: we need more ecological data, we need amateurs and professionals to build networks, and we need to survey endangered habitats with the utmost urgency. The task at hand is enormous, but with greater coordination and a shared sense of purpose, amateur and professional mycologists can make great strides toward documenting, understanding, and hopefully conserving fungal diversity.



[back to top](#)

ACKNOWLEDGMENTS

I would like to thank Dr. Else Vellinga for her insightful comments on an earlier draft of the manuscript, and four reviewers for reading the manuscript prior to final submission.

REFERENCES

- Agerer, R. 1999. Never change a functionally successful principle: The evolution of *Boletales* s.l. (*Hymenomycetes*, *Basidiomycota*) as seen from below-ground features. *Sendtnera* 6: 5-92.
- Aguilera, G., S. Marthey, H. Chiapello, M.-H. Lebrun, F. Rodolphe, E. Fournier, A. Gendrault-Jacquemard, and T. Giraud. 2008. Assessing the performance of single-copy genes for recovering robust phylogenies. *Systematic Biology* 57(4): 1-15.
- Arora, D. 1986. *Mushrooms Demystified*, 2nd edition. Berkeley, CA: Ten Speed Press. 1020 pp.
- Bunyard, B.A. 2009. Whither the field mycologist? *Fungi* 2(3): 35-39.
- Gryta, H., J.-C. Debaud, A. Effosse, G. Gay, and R. Marmeisse. 1997. Fine-scale structure of populations of the ectomycorrhizal fungus *Hebeloma cylindrosporum* in coastal sand dune forest ecosystems. *Molecular Ecology* 6: 353-364.
- Halling, R.E., T.J. Baroni, and M. Binder. 2007. A new genus of Boletaceae from eastern North America. *Mycologia* 99(2): 310-316.
- Henkel, T., M.C. Aime, and S.L. Miller. 2000. Systematics of pleurotoid *Russulaceae* from Guyana and Japan, with notes on their ectomycorrhizal status. *Mycologia* 92: 1119-1132.
- Hennig W. 1966. *Phylogenetic Systematics*. Urbana: University of Illinois Press. 263 pp.
- Hesler, L.R. 1969. *North American Species of Gymnopilus* (Mycologia Memoir No. 3). New York: Hafner Publishing Company. 117 pp.
- Hibbett, D.S., E.M. Pine, E. Langer, G. Langer and M.J. Donoghue. 1997. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. *Proceedings of the National Academy of Sciences* 94: 12002-12006.
- Hibbett, D.S., L-B. Gilbert and M.J. Donoghue. 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature* 407: 506-508.
- Hofstetter V, Cléménçon H, Vilgalys R, Moncalvo J-M. 2002. Phylogenetic analyses of the *Lyophylleae* (*Agaricales*, *Basidiomycota*) based on nuclear and mitochondrial rDNA sequences. *Mycological Research* 106 (9): 1043-1059.
- Kendrick, B. 1994a. Evolution in action: from mushrooms to truffles? (Part 1). *Mcllvainea* 11(2) 34-38.
- Kendrick, B. 1994b. Evolution in action: from mushrooms to truffles? (Part 2). *Mcllvainea* 11(2) 39-47.
- Korf, R.P. 2005. Reinventing taxonomy: a curmudgeon's view of 250 years of fungal taxonomy, the crisis in biodiversity, and the pitfalls of the phylogenetic age. *Mycotaxon* 93: 407-415.
- Kuo M. 2007. Mushrooming in the age of DNA: now comes the fun part. *Mcllvainea* 17(1): 43-49.
- Lange, M. and F.B. Hora, 1963. *Collins Guide to Mushrooms and Toadstools*. London: Collins. 257 pp.
- Leonard, L. 2008. [Letter to the Editor]. Reply to: "Mushrooming in the age of DNA: now comes the fun part." *Fungi* 1(1): 1.
- Luttrell, E.S. 1958. The function of taxonomy in mycology. *Mycologia* 50(6): 942-944.
- Mani, G.S. and B.C. Clarke. 1990. Mutational order: a major stochastic process in evolution. *Proceedings of the Royal Society of London, Series B: Biological Sciences* 240(1297): 29-37.
- Martin, F., A. Aerts, D. Ahren, A. Brun, E.G.J. Danchin, F. Duchaussoy, J. Gibon, A. Kohler, E. Lindquist, V. Pereda, A. Salamov, H. J. Shapiro, J. Wuyts, D. Blaudez, M. Buée, P. Brokstein, B. Canbäck, D. Cohen, P. E. Courty, P. M. Coutinho, C. Delaruelle, J. C. Detter, A. Deveau, S. DiFazio, S. Duplessis, L. Fraissinet-Tachet, E. Lucic, P. Frey-Klett, C.

- Fourrey, I. Feussner, G. Gay, J. Grimwood, P. J. Hoegger, P. Jain, S. Kilaru, J. Labbé, Y. C. Lin, V. Legué, F. Le Tacon, R. Marmesse, D. Melayah, B. Montanini, M. Muratet, U. Nehls, H. Niculita-Hirzel, M. P. Oudot-Le Secq, M. Peter, H. Quesneville, B. Rajashekar, M. Reich, N. Rouhier, J. Schmutz, T. Yin, M. Chalot, B. Henrissat, U. Kües, S. Lucas, Y. Van de Peer, G. K. Podila, A. Polle, P. J. Pukkila, P. M. Richardson, P. Rouzé, I. R. Sanders, J. E. Stajich, A. Tunlid, G. Tuskan and I. V. Grigoriev. 2008. The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature* 452(7183): 88-92.
- McAdam, A. 2007. Keys to the British genera of agarics and boleti. British Mycological Society. (URL: http://www.britmycolsoc.org.uk/files/KEYS_TO_THE_BRITISH_GENERA_OF_AGARICS_AND_BOLETI_REVISIED_FEBRUARY_2009.pdf)
 - Miller, O.K. Jr., T. Henkel, T.Y. James, and S.L. Miller. 2001. *Pseudotulostoma*, a remarkable new volvate genus in the *Elaphomycetaceae* from Guyana. *Mycological Research* 105: 1268-1272.
 - Miller, S.L., T.M. McClean, J.F. Walker and B. Buyck. 2001. A molecular phylogeny of the *Russulales* including agaricoid, gasteroid and pleurotoid taxa. *Mycologia* 93(2): 344-354.
 - Morel, M., C. Jacob, A. Kohler, T. Johansson, F. Martin, M. Chalot, and A. Brun. 2005. Identification of genes differentially expressed in extraradical mycelium and ectomycorrhizal roots during *Paxillus involutus*-*Betula pendula* ectomycorrhizal symbiosis. *Applied and Environmental Microbiology* 71:382-391.
 - Mueller, G.M. 1992. Systematics of *Laccaria* (Agaricales) in the Continental United States and Canada, With Discussions on Extralimital Taxa and Descriptions of Extant Types. *Fieldiana: Botany*, n.s., 30: 1-158. (URL: http://www.fieldmuseum.org/research_collections/botany/botany_sites/fungi/index.html; navigate to the section "Intro," then the link "Phylogenetic Considerations" to view the results of the phylogenetic analysis).
 - Mueller, G.M. and E.M. Pine. 1994. DNA data provide evidence on the evolutionary relationships between mushrooms and false truffles. *Mycologia* 11(2): 61-74.
 - Nygren, C. M. R., U. Eberhardt, M. Karlsson, J. L. Parrent, B. D. Lindahl, and A. F. S. Taylor. 2008. Growth on nitrate and occurrence of nitrate reductase-encoding genes in a phylogenetically diverse range of ectomycorrhizal fungi. *New Phytologist* 180(4): 875-889.
 - Osmundson, T.W., C.L. Cripps, and G.M. Mueller. 2005. Morphological and molecular systematics of Rocky Mountain alpine *Laccaria*. *Mycologia* 97(5): 949-972.
 - Peintner, U., N. L. Bougher, M. A. Castellano, J.-M. Moncalvo, M. M. Moser, J. M. Trappe, and R. Vilgalys. 2001. Multiple origins of sequestrate fungi related to *Cortinarius* (*Cortinariaceae*) *American Journal of Botany* 88: 2168-2179.
 - Rokas, A., B.L. Williams, N. King, and S.B. Carroll. 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature* 425: 798-804.
 - Saenz, G.S. 1998. Evolutionary relationships of the powdery mildews (*Erysiphales*) inferred from ribosomal DNA sequences. *Mycologia* 13(2): 33-44.
 - Saenz, G.S. and J.W. Taylor. 1999. Phylogeny of the Erysiphales (powdery mildews) inferred from internal transcribed spacer ribosomal DNA sequences. *Canadian Journal of Botany* 77(1): 150-168.
 - Schluter, D. 2009. Evidence for ecological speciation and its alternative. *Science* 323: 737-741.
 - Singer, R. 1986. *The Agaricales in Modern Taxonomy*, 4th edition. Koeltz Scientific Books, Königstein, Germany.
 - Sutara, J. (1992). The genera *Paxillus* and *Tapinella* in Central Europe. *Ceska Mykologia* 46: 50-56.
 - Vilgalys, R., A. Smith, B.L. Sun, and O.K. Miller, Jr. 1993. Intersterility groups in the *Pleurotus ostreatus* complex from the continental United States and adjacent Canada. *Canadian Journal of Botany* 71: 113-128.