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A critique of DNA sequence analysis in the taxonomy of filamentous Ascomycetes and ascomycetous anamorphs

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Abstract: The validity of reclassifying filamentous, ascomycetous anamorphs solely on the basis of ribosomal DNA sequences is examined. We suggest that emotional reactions to the debate are a consequence of often unacknowledged philosophical biases. From the perspective of the scientific method, neither morphological nor sequence-based taxonomic studies are inherently superior. A review of published information on the internal transcribed spacer of filamentous Ascomycetes and ascomycetous anamorphs demonstrates that uniform species concepts based on DNA sequences alone are presently infeasible. Because a phylogenetic scheme should classify species, the concept that fungi can be typified or classified solely by DNA sequences is challenged. Similarly, because no adequate nonmorphological species concept exists for anamorphic fungi that lack a sexual state, integration of the Deuteromycetes into the holomorphic classification on the basis of DNA sequences alone is also presently impractical.

Key words: DNA sequencing, fungal taxonomy, internal transcribed spacer, species concepts.

Résumé : Les auteurs examinent l'intérêt de reclassifier les ascomycètes anamorphes filamenteux sur la seule base des séquences de l'ADN ribosomique. Ils suggèrent que les réactions émotives au débat sont la conséquence de biais philosophiques le plus souvent non-déclarés. Dans une perspective de méthodologie scientifique, les études taxonomiques, ni la morphologie, ni les séquences ne sont supérieures de façon inhérente. Un survol des informations publiées concernant l'espaceur interne transcrit chez les ascomycètes filamenteux et chez les ascomycètes anamorphes démontre que des concepts uniformes d'espèces basés sur les seules séquences de l'ADN sont pour le moment irréalisables. Parce qu'un schème phylogénétique devrait classifier les espèces, le concept selon lequel les champignons pourraient être caractérisés ou classifiés à partir des seules séquences de l'ADN est contesté. De même, parce qu'il n'existe pas de concept d'espèce non-morphologique pour les champignons anamorphes sans stade sexuel connu, l'intégration des deutéromycètes dans la classification holomorphe sur la seule base des séquences d'ADN est également considérée comme impraticable.

Mots clés : séquences d'ADN, taxonomie fongique, espaceur interne transcrit, concepts d'espèces.
[Traduit par la rédaction]

Introduction

> "The ultimate aim of the modern movement in biology is in fact to explain *all* biology in terms of physics and chemistry."
>
> Francis Crick, 1966
> *Of Molecules and Men*

In mycological taxonomy, the rift between traditional morphologists and molecular phylogeneticists remains unbridged. No one would deny that the debate reflects different approaches to science, but how deeply the controversy is rooted seems hardly to have been considered. Part of the problem is education. Until recently, it was rare to find individuals who were conversant with both approaches. A comparatively small number of systematists have demonstrated insight and sophistication with both kinds of characters. The majority of fungal taxonomists, although they may proclaim an understanding of both sets of techniques, find their true sympathies (and demonstrated competencies) on one side or the other. The simultaneous adoption of cladistic techniques and terminology by sequencing systematists may have further alienated traditional morphologists in their struggle to stay current with contemporary taxonomic thought.

The papers in this supplement of the Canadian Journal of Botany from the section Classifying sexual and asexual fungi: do we need the Deuteromycetes? follow a volume of similar papers edited by Reynolds and Taylor (1993). In this paper, we examine a corollary of the rhetorical question posed by the originators of this debate. If the Deuteromycetes are to be abandoned because a unified classification of all fungi may be possible from DNA sequences (Bruns et al. 1991;

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Reynolds and Taylor 1991), we must prove that fungi really can be classified by DNA sequences alone. Our presentation tests this hypothesis, concentrating on work published on filamentous Ascomycetes and ascomycetous anamorphs. Our arguments will return to the original question of the need for the Deuteromycetes near the end of the paper.

Philosophical considerations

Few working scientists consider the philosophical biases they bring to their work. As the quote at the start of this paper suggests, there is an implicit philosophical undercurrent to much of modern biology, particularly molecular biology. The mechanistic or materialist view of living things seeks to explain all aspects of biology in terms of molecules undergoing chemical reactions according to physical laws; in short, the organism as a machine. The contrary view, that there is an essence of life that is somehow beyond the realms of physics and chemistry, is known as vitalism. These concepts have been debated for 2000 years, since vitalism was expounded by Plato and mechanism by his student, Aristotle (see review in Lewin 1992).

Vitalism, because of its spiritual or metaphysical emphasis, has been firmly rejected by 20th century science. By default, it seems, biological materialism now dominates, although few scientists realize there are alternatives. Reductionism is a component of both materialism and of the scientific method and the two are often confused. The idea that the evolutionary history of an organism can be understood by a genetic sequence alone is surely biological materialism; that it can be understood by a partial sequence of one gene is also highly reductionistic. Reductionist tendencies in modern genetics have been criticized at length, for example by Lewontin (1991).

Despite the rejection of vitalism by much of modern science, vitalistic viewpoints are evident in some morphological taxonomy. For example, vitalistic concerns may be reflected in taxonomic concerns about how representative herbarium specimens, cultures, or dead cells examined through the microscope are of living organisms in nature (Baral 1992). Furthermore, current interest in nonlinear dynamics and emergent phenomena (also known as complexity theory) has led to the development of what has been called neovitalism (Waldrop 1992; Lewin 1992). Neovitalism removes the mystical components of traditional vitalism, viewing living organisms as complex systems that obey the laws of physics and chemistry but live along the edge of chaos, rendering their behaviour unpredictable.

If philosophical predispositions are not acknowledged or understood, debates between morphological and molecular systematists may never be resolved. The differences are reflected in many ways, in the debates over whether genotypic or phenotypic are most appropriate for phylogenetic analysis, and whether cladistics (a strictly linear analysis) is superior to so-called intuitive analysis (an emphatically nonlinear analysis). We will touch on these issues below, pointing out the fingerprints of biological materialism, vitalism, and neovitalism where we find them.

Sequence-based systematics and the scientific method

Philosophical disagreements between morphological and molecular systematists obscure whether one approach is scientifically superior to the other. If materialism is confused with the scientific method, one might assume that sequence-based systematics is more scientific than morphological systematics. Discrete, quantitative characters are assumed to be more scientific than sometimes fuzzy, qualitative characters. Computerized cladistic analysis is assumed to be more scientific than noncomputerized, intuitive analysis. Is this really true or is technology being confused with science?

The scientific method consists of three interconnected steps: (*i*) observation, (*ii*) hypothesis formation, and (*iii*) experimentation (Baird 1962). For the first two steps of the scientific method, however, there is little difference in scientific validity between morphological and sequence-based systematics.

It is worth noting the primacy of observations in the scientific method. Most published biology, especially in morphological studies, consists of reports of observations. Is one method of gathering observations more valid than another? As long as observations are reproducible, the method used to obtain them is unimportant. Levels of resolution in biological observation are made possible by technology. Observations made with the naked eye are less detailed than those made with optical equipment. In turn, these are less detailed than those made with electron microscopes. Sequences of proteins and nucleic acids generated by molecular biologists represent the most detailed observations in biology. In general, as observations become more specific, they give a more detailed representation of the genotype but represent less of the phenotype. The totality of the living organism is easily overlooked as observations become more detailed, a criticism often made of techno-taxonomy by traditional taxonomists.

DNA sequence data are often considered unequivocally correct observations but their reproducibility is rarely rigorously tested. Most sequences have unreadable bases resulting from peculiarities in secondary DNA structure, DNA polymorphisms, and conformational limitations of sequencing enzymes. The number of unreadable bases sometimes comprises a significant ratio with the number of bases differing between closely related taxa. It is considered good genetic practice to read both strands of the DNA but this is not always done. Bases that remain unreadable after sequencing both strands might be resolved using a different sequencing enzyme and (or) protocol, but few taxonomic laboratories go to this extreme. Interpretation of sequencing gels requires experience, a similar amount of experience, perhaps, to learning to recognize a particular type of conidiogenous cell through the microscope.

Taxonomic concepts and classifications are hypotheses (Luttrell 1977), so most taxonomic studies fulfil the second criterion of the scientific method. The scientific method does not dictate how hypotheses should be derived. Hypotheses generated by the statistical ruminations of computer programs may appear more scientific than those generated from an experienced taxonomist's mind, a subject we will revisit below. Many biological studies are undertaken with the working hypothesis that something will be discovered, rather than as tests of particular hypotheses. A computerized phylogenetic or phenetic analysis performed simply to see how things fall out is no more inherently scientific than a so-called intuitive analysis, no matter what its repeatability.

The scientific completeness of taxonomic studies usually

depends on whether experiments are planned, executed, and replicated to confirm or reject a hypothesis. In practice, taxonomic studies are rarely overtly experimental in nature, and this is equally true for morphological and sequencing studies. Many experimental taxonomic projects employing sequencing are designed to test classification hypotheses already formulated by morphological taxonomists (Berbee and Taylor 1992; Saenz et al. 1994). Kohn (1992) has described how the null hypothesis can be incorporated in taxonomic studies, irrespective of the methods employed, so that the requirements of the scientific method are fulfilled.

Traditionally, the experimental test of classification hypotheses has been the province of the user community. Other scientists attempt to use the proposed taxonomies and fit other, previously unknown taxa into the established scheme. At the moment, the utilitarian aspect of morphological systematics is its one big advantage over sequence-based systematics. Morphological studies are used for identifications, and hence the validity of the hypotheses and the reproducibility of the observations are tested every time someone tries to identify a fungus. With time, this global taxonomic experiment refines the taxonomic hypotheses inherent in a classification, just as the reliability and reproducibility of any scientific hypothesis is tested. At present, sequence-based systematics is directed mostly towards recognition of monophyletic groups, and not the other aspect of the taxonomic mandate, identification. Therefore, the hypotheses and observations are only tested by laboratories that have the technological and financial resources to do so. However, those of us who do large numbers of routine identifications keenly anticipate the time when sequence- or probe-based identification kits are available for common fungi.

Sequence-based systematics as it is practiced

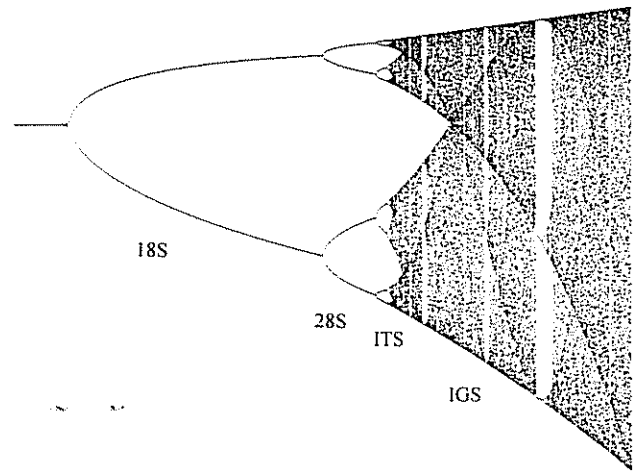
What is the significance of a DNA sequence in the larger context of the whole organism? Taxonomic theory warns that classifications should not be based on single characters. To a morphologist, a ribosomal RNA gene sequence is seen as equivalent to a single character, simply a highly dissected character. In an analysis of sequence data, each nucleotide base is considered a character with four possible character states. These different perceptions are a major component of the disagreement between the two sides.

Morphologists often complain that extraordinary taxonomic weight is given to the sequence of a single gene, which represents a fraction of the part of the genome sampled by a morphological description. We do not know how many genes are involved with most morphological characters. It is safe to assume that there is at least an order of magnitude more information sampled in a morphological trait such as conidium ontogeny than there is in a ribosomal RNA gene sequence. Genes involved with anamorphic morphology have been studied in *Neurospora crassa* (13 known regulatory genes involved in conidium ontogeny; Roberts and Yanofsky 1989) and *Emericella nidulans* (14 known genes involved in initiation and development of conidiophores, phialides, and conidia; Timberlake 1990).

Selecting the appropriate region for study

Most sequence-based systematics undertaken with fungi focuses on nuclear and mitochondrial ribosomal RNA genes,

Fig. 1. The chaotic bifurcation diagram as a metaphor for a cladogram and taxonomic resolution of different regions of the ribosomal DNA genes. To the left of the diagram, the higher level taxa are generally clearly resolved, but towards the right, results of sequence analysis may be equivocal. Do they fall into the realm of taxonomic chaos? Compare with the cladogram with unresolved terminal branches in Avise 1994, p. 5 (diagram prepared using the program FractInt (Wegner et al. 1992)).



for reasons reviewed by Bruns et al. (1991). They are present and homologous in all cellular organisms, and exist in a large number of copies in each cell, which simplifies detection and isolation. The ribosomal gene has several coding and noncoding regions that evolve at different rates, allowing their sequences to be exploited at different taxonomic levels. For example, the 18S (or nuclear small subunit) gene often is used for studying relationships at or above the generic level. The 28S (or nuclear large subunit) gene, particularly the so-called D2 domain, may be useful for studying relationships near the generic level. The internal transcribed spacer (ITS, often subclassified into ITS1 and ITS2, two regions separated by the 5.8S subunit), and to a lesser extent the mitochondrial (16S) small subunit gene, are used for studying relationships near the species level. Presented this way, the question of which region to sequence for a particular study seems simple. In fact, these statements represent hypotheses formulated by sequencing systematists (Fig. 1). They are tested repeatedly, much as the utility of certain morphological characters is tested by the morphological community.

In recent years, interpretation of ribosomal RNA genes has become more complicated. As Bruns et al. (1991) note, a taxonomically ideal molecule would exist in or behave as a single copy, so that it would be certain that homologous genes were being compared. Careful studies have revealed different versions of ribosomal genes within single genomes or among strains in several groups of fungi (e.g., Garber et al. (1988) for *Cochliobolus*, O'Donnell (1992) for *Fusarium*, Martin (1990) for *Pythium*). The increasing recognition of rRNA gene heterogeneity suggests that it is no longer adequate to sequence a single strain of a species in a taxonomic study. It also suggests that DNA sequences from polymerase chain reaction (PCR) products should be done in duplicate, a practice that is rarely followed.

The 18S gene stands up well as an indicator of higher fungal relationships, although some conclusions at the ordinal

Table 1. Base differences reported among isolates of a single species in the nuclear ITS and 28S ribosomal RNA gene regions.

Taxon	Region	Bases	No. of differences	% difference	Ref.
	ITS				
Penicillium duclauxii (2)	ITS1-2	913	0	0	24
Penicillium vulpinum (2)	ITS1-2	913	0	0	24
Penicillium clavigerum (2)	ITS1-2	913	0	0	24
Botrytis cinerea (3)	ITS1	200	0	0	9
Sclerotinia sclerotiorum (3)	ITS1	200	0	0	9
Sclerotinia trifoliorum (3)	ITS1	200	0	0	9
Sclerotinia minor (3)	ITS1	200	1	0.5	9
Metarhizium anisopliae (11)	ITS1-2	601	38	6.3	12
Colletotrichum kahawae (10)	ITS1	170	0	0	43
Colletotrichum gloeosporioides (5)	ITS1	170	3	1.8	43
Colletotrichum acutatum (12)	ITS1	170	12	7	44
Colletotrichum orbiculare (12)	ITS2-28S	886	40	4.5	41
Leptosphaeria korrae (2)	ITS1	249	13	5.2	15
Beauveria brongniartii (7)	ITS1-2	838	88	10.5	29
Seiridium cardinale (12)	ITS1	183	29	15.8	48
	28S				
Gibberella fujikuroi varieties (6)	28S-D2	220	0	0	33
Gibberella pulicaris (7)	28S-D2	220	1	0.5	33

NOTE: In parentheses following the species name are the number of isolates compared.

and family levels have been controversial. The hotly debated hypothesis of the derivation of Ascomycetes from red algae has been convincingly rebuked. The close relationship of the Chytridiomycetes to other fungi and the chromistan relationships of the Oomycetes have been clarified by studies of 18S rRNA gene sequences (Bruns et al. 1991). The separation of *Ophiostoma* and *Ceratocystis*, supported by anamorph characters, cell wall composition, and antiobiotic resistance, also has been supported by 18S ribosomal RNA gene sequence data (Spatafora and Blackwell 1994; Hausner et al. 1993).

The species enigma

What constitutes a species remains a major taxonomic problem, regardless of methodology (Mayr 1988). Species concepts also are critical to the hypothesis that fungi, particularly anamorphic fungi, might be classified by DNA sequences alone. Many species concepts are employed by taxonomists. The biological species concept, in which members of a species must be able to interbreed sexually, has been widely adopted by mycologists in recent years.

Most biologists were taught that the species is the unit of evolution, the one taxonomic category not created by man. Strict materialistic interpretations of evolution might reject this special status for the taxonomic rank of species. In any case, taxonomic systems classify species, not individuals. Is contemporary sequence-based systematics successful in this regard?

Morphologists often ask how many base differences are required for strains to be considered different species. In a phylogenetic analysis, cladogram topology indicates monophyletic groups that may represent species or supra- or subspecific taxa. Sequence-based systematists sometimes express an intuitive feel for how much sequence divergence indicates separate species (e.g., quote by Peterson on p. 355 of Logrieco et al. 1990). Recognition of species from sequence data is challenging, because there must be enough variation to distinguish taxa, but not so much that relationships are obscured. In Tables 1 and 2, we compare the amount of sequence divergence within a species, or among closely related species, reported for the ITS and 28S subunits in various independent studies, using the species concepts accepted by the authors of those studies. To date, insufficient variation has been demonstrated in the 28S subunit gene to allow the consistent resolution of different species of filamentous ascomycetous fungi (Table 2), although the region is considered useful for yeasts (Kurtzman 1993). Therefore, it is premature to rely on this domain for satisfactory sequence-based species concepts.

The ITS region has been studied more extensively. As Table 1 shows, there is little reported variation in parts of the ITS for some species, but up to about 15% variation within other species. The data indicates a tendency for the observed sequence variation within a species to increase with the number of strains of that species studied. A further complication is that the number of base differences between purported sibling species pairs shows a similar variation to that sometimes seen within single species (Table 2). Clearly, the rate of evolution of the ITS region is variable in the fungi and the molecular clock concept applies only within a closely related group of species. A similar conclusion was drawn by Taylor et al. (1990), who tried to correlate nuclear ITS and mitochondrial small ribosomal gene nucleotide divergence with genera and species in the Agaricales and Sordariales and wrote "that the amount of nucleotide divergence cannot be used to define taxonomic rank throughout the higher fungi, and probably not within even a single order."

Cladistic concepts require only that species represent monophyletic groups (Wiley et al. 1991) and offer no guidelines on how to rank individuals. All acceptable taxa in a

Table 2. Base differences in the nuclear ITS and 28S ribosomal RNA gene regions between species pairs.

Taxon pairs	Region	Bases	No. of differences	% difference	Ref.
	ITS				
Sclerotium sclerotiorum/minor	ITS1	200	0 or 1	0–0.5	9
Sclerotium sclerotiorum/trifoliorum	ITS1	200	1	0.5	9
Sclerotium minor/trifoliorum	ITS1	200	1 or 2	0.5–1	9
Beauveria brongniartii/bassiana	ITS1-2	838	6	0.7	29
Verticillium dahliae/alboatrum	ITS1-2	543	5	0.9	28
Verticillium dahliae/tricorpus	ITS1-2	543	17	3.1	28
Verticillium albo-atrum/tricorpus	ITS1-2	543	12	2.2	28
Colletotrichum gloeosporioides/kahawae	ITS1	170	4	2.4	43
Colletotrichum gloeosporioides/musae	ITS1	170	12	7	44
Colletotrichum gloeosporioides/acutatum	ITS1	170	28	16.5	44
Colletotrichum acutatum/musae	ITS1	170	26	15.3	44
Metarhizium anisopliae/flavoviride	ITS1-2	601	75	12.5	12
Metarhizium anisopliae/album	ITS1-2	601	86	14.3	12
Metarhizium album/flavoviride	ITS1-2	601	94	15.6	12
Hymenoscyphus monotropae/ericae	ITS1	292	48	16	13
	28S				
Gibberella fujikuroi var. *moniliformis/fujikuroi*	28S-D2	220	2	0.9	33
Gibberella fujikuroi var. *moniliformis/intermedia*	28S-D2	220	2	0.9	33
Gibberella fujikuroi var. *fujikuroi/intermedia*	28S-D2	220	0	0	33
Penicillium chrysogenum/aethiopicum	28S-D2	245	0	0	32
Penicillium crustosum/roquefortii	28S-D2	245	0	0	32
Penicillium commune/expansum	28S-D2	245	0	0	32
*Penicillium expansum/thomii**	28S-D2	245	16	6.5	32
*Penicillium expansum/aculeatum**	28S-D2	245	48	19	32

NOTE: Where infraspecific variation has been demonstrated for any of the taxa compared, only differences not found in any isolates of the compared species are counted.

*Species pairs in different subgenera.

phylogenetic classification will be monophyletic. How does a species differ from the rest of them? The ranking of monophyletic groups often depends on contextual data independent of a sequence. The significance of the number of base differences and which clades represent species has to be calibrated by considering infraspecific variation, as well as interspecific variation of closely related fungi that are known to be different biological or otherwise species.

While a universally applied species concept based on ITS sequences is infeasible, carefully designed studies of the ITS are beginning to help resolve species complexes in monophyletic taxa. Resolution of species within the *Colletotrichum gloeosporioides* complex, for example, has long been problematic because morphologists could not agree on how to interpret morphological variation and host relationships. Despite the existence of considerable variation within the ITS2-28S of *C. orbiculare*, a phenetic analysis of sequence data was compatible with morphological observations of conidium septation, and the strains were contained within a well-supported cluster (Sheriff et al. 1994). The ITS1 sequence of *Colletotrichum kahawae* was uniform for 10 strains originating on coffee plants from several African countries (Sreenivasaprasad et al. 1993). Similarly, a molecular phylogeny of insect pathogens in the genus *Metarhizium* correlates well with the accepted taxonomy (Curran et al. 1994), despite a high amount of heterogeneity in the ITS sequences, supporting the concept that the morphologically defined species represent monophyletic groups.

Some species complexes have so far not been resolved using the ITS. Carbone and Kohn (1993) found very little variation in the ITS1 region between sibling species of the *Sclerotium sclerotiorum* complex. Egger and Sigler (1993) were unsuccessful in their attempt to use ITS sequences to infer an anamorph–teleomorph connection between the North American *Vaccinium* endophyte, *Scytalidium vaccinii*, and the European taxon *Hymenoscyphus ericae*. Although the taxa were closely related in a phylogenetic analysis, it was impossible to decide whether they were conspecific without supporting mating data. Neuvéglise et al. (1994) recognized seven sequence groups among 30 strains of *Beauveria brongniartii*, but were unable to satisfactorily rank any of these groups as species, or to fully differentiate them from strains identified as *B. bassiana*.

This returns us to the question of the abandonment of the Deuteromycetes. Biological species concepts cannot be applied to organisms that do not undergo meiosis. Sequence-based species concepts may have very limited applicability. Perkins (1991) has proposed that anamorphic species not be consid-

ered species at all. He suggests we use another term for them, and reserve the term species for organisms that undergo meiosis. We have two reactions to this idea. First, it echoes the dismissive treatment anamorphic fungi often receive from those who prefer to ignore rather than accommodate them. Second, whether these taxa are called species or not, they must still be classified, and we must develop concepts that allow us to group like with like. In the absence of a workable species concept, how can the Deuteromycetes be reclassified in the holomorphic system on the basis of DNA sequences alone?

Data analysis

Reproducible data analysis is one of the strongest contributions sequence-based systematics has made to mycological taxonomy. Most analyses have been cladistic, but sometimes phenetic analyses are used for sequence data (e.g., Sherriff et al. 1994). Despite the obvious scientific merit in employing reproducible data analysis, it is safe to say that much of the unease about sequence-based systematics felt by traditional taxonomists comes from a distrust of computerized analysis. The distrust is partly a result of ignorance, an inability or unwillingness to penetrate the terminological fog that often surrounds cladistic arguments. The designation of cladistics as phylogenetic systematics is considered pretentious by some, because it implies that other taxonomic analyses are not phylogenetic. But the major reason traditional morphologists have for distrusting cladistics, and other forms of computerized data analysis, is more complex, and firmly bound in philosophy.

For the biological materialist, seeking to understand an organism by dissecting its parts, computerized analysis makes perfect sense. Congruence between two cladograms, derived from sequences of different genes (LoBuglio et al. 1993; Tasi et al. 1994), constitutes strong statistical support of the hypothetical phylogeny. To the vitalist or neovitalist, seeking to obtain a holistic understanding of an organism, computerized analysis is less impressive. A DNA sequence is considered too trivial to be representative of all characters. Genotypic statistics that can't be related to phenotypic characters are considered unimpressive. And there is the realization that no computer can produce an analysis as sophisticated as that provided by the human brain. As has been stated by Hillis (quoted in Lewin 1992), the most sophisticated computers in the world are "trivial in complexity compared with the brain of a fly."

Intuitive analysis is the somewhat dismissive term that has been used to characterize taxonomic analyses that do not employ computers. Traditional taxonomists are often accused of ignoring data if they question the results of computerized analyses. In some cases, they retreat behind the screen of authority, a defensive position that some have criticized as unscientific (Williams and Round 1994). We suggest that experience plays a real but unmeasurable role in all scientific disciplines, and that intuition is often the reflection of that experience. Given the complexity of the human brain, the volume of data and analysis inherent in any intuitive judgement should be routinely dismissed only by the naive.

We suggest that phylogenies, whatever the origin of the data and the type of analysis that produce them, require a demonstrable link to phenotypic characters before they can be widely accepted. This does not mean that they must conform to historically developed classifications, only that they be consistent with the biology of the organisms and the world in which they evolved.

Revitalizing morphology

It is hard to believe that anyone connected with fungal systematics could believe that morphology has run its course. The arguments in support of morphology have been presented *ad nauseam*, and the opinions of one of us are included in the proceedings of the Holomorph Conference (Seifert 1993). The problem is not that morphology is unscientific or irrelevant, but that the data set is so incomplete, and has been explored only in the most basic of ways. Morphological systematists are partly to blame for their own problems. A lack of standardized, stable terminology is a perpetual problem. The tendency to engage in monothetic taxonomy has led to quite accurate charges of subjectivity. The pseudo-philosophical debates between so-called lumpers and splitters have tainted the descriptions and illustrations taxonomists provide. How often have morphological observations been filtered through the preconceived biases of the taxonomist deciding what is worth reporting? We have a lot to learn from sequencing systematists about objectivity and reproducible data analysis.

Our impression is that morphologists throw too much information away. This is partly because of a lack of mental or electronic processing power to handle the reams of potential data, and partly a lack of awareness that the information even exists. We call phenotypic characters that are subconsciously perceived but not articulated subliminal characters. It is subliminal characters, never described or quantified, that allow experienced taxonomists to accurately identify microscopic fungi using a dissecting microscope, without observing any of the diagnostic characters. Three-dimensional perception undoubtedly plays as role, but most structures are measured, depicted, and analyzed as if they have only two dimensions. Other subtle aspects of texture, shape, and colour, and interactions between the three, no doubt are also important.

Fortunately, modern technology offers some solutions and the possibility of a renaissance in morphological studies. Image analysis allows the automated quantification of shapes, sizes, and colours of relatively large numbers of structures. Electronic particle sizing offers the possibility of measuring size, volume, DNA, and lectin contents of about 30 000 spores in 1–2 min (Chapela 1991). Finally, fractal geometry may have a lot to offer in the analysis of fungal morphology. The conidiophores of *Trichoderma* species, for example, which have long defied precise description, are similar to tree-like structures derived from mathematical formulae called iterated functional systems, or so-called L-systems (Prusinkiewicz and Lindenmayer 1990).

In this paper, we have emphasized the philosophical differences that underlay the disagreements between traditional morphological and molecular systematics. We have concluded that neither is necessarily more compatible with the scientific method. We have emphasized that a phylogenetic scheme should classify species, and that recognizing species from DNA sequences is sometimes problematic.

The question is often asked whether a person trained as a

classical taxonomist can also do molecular biology. Anybody who has done both kinds of work knows that they exploit different aptitudes, that different parts of the brain are used for the microscope and for the propipetter. It will be a rare individual who is equally talented with both. One solution to variable competency is the formation of multidisciplinary teams. Given the funding void for taxonomic studies, these are likely to be assembled only in exceptional circumstances. It behooves the rest of us to apply the scientific method using whatever techniques are at hand.

As an example of taxonomic chaos, but hardly an unusual one, the history of the Dutch Elm disease fungus is a frightening tale (Brasier 1993) . It was first described as an anamorph, *Graphium ulmi*, by Schwarz (1922). Its teleomorph was described in *Ceratostomella* 10 years later (Buiseman 1932). The species was passed from *Ophiostoma* to *Ceratocystis* and back to *Ophiostoma* again over a 60-year period. In the 1990s, when *Ophiostoma* was finally established as the appropriate genus for the fungus, Brasier (1991) described a new species, *O. novo-ulmi*, the cause of the current pandemic of Dutch elm disease. It is uncertain whether *O. novo-ulmi* is newly evolved, or whether it is a previously extant population that suddenly exploited a newly available niche. In *Ophiostoma*, at least, we have to consider the possibility that fungi are evolving faster than our ability to classify them. We have to recognize and accept our philosophical differences and resume the job at hand, otherwise we will never catch up.

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References

1. Avise, J.C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.
2. Baird, D.C. 1962. Experimentation: an introduction to measurement theory and experimental design. Prentice-Hall Inc., Englewood Cliffs, N.J.
3. Baral, H.O. 1992. Vital versus herbarium taxonomy: morphological differences between living and dead cells of Ascomycetes and their taxonomic implications. Mycotaxon, **44**: 333–390.
4. Berbee, M.L., and Taylor, J.W. 1992. 18S ribosomal RNA gene sequence characters place the human pathogen *Sporothrix schenckii* in the genus *Ophiostoma*. Exp. Mycol. **16**: 87–91.
5. Brasier, C.M. 1991. *Ophiostoma novo-ulmi* sp. nov., causative agent of the current Dutch elm disease pandemics. Mycopathologia, **115**: 151–161.
6. Brasier, C.M. 1993. The genetic system as a fungal taxonomic tool: gene flow, molecular variation and sibling species in the '*Ophiostoma piceae* – *Ophiostoma ulmi*' complex and its taxonomic and ecological significance. *In Ceratocystis* and *Ophiostoma*, taxonomy, ecology, and pathogenicity. *Edited by* M.J. Wingfield, K.A. Seifert, and J.F. Webber. American Phytopathological Society, St. Paul, Minn. pp. 77–92.
7. Bruns, T.D., White, T.J., and Taylor, J.W. 1991. Fungal molecular systematics. Annu. Rev. Ecol. Syst. **22**: 525–564.
8. Buiseman, C. 1932. *Ceratostomella ulmi*, de geslachtelijke vorm van *Graphium ulmi* Schwarz. Tijdschr. Plantenziekten, **38**: 1–5.
9. Carbone, I., and Kohn, L.M. 1993. Ribosomal DNA sequence divergence within internal transcribed spacer 1 of the Sclerotiniaceae. Mycologia, **85**: 415–427.
10. Chapela, I.H. 1991. Spore size revisited: analysis of spore populations using an automated particle sizer. Sydowia, **43**: 1–14.
11. Crick, F. 1966. Of molecules and men. University of Washington Press, Seattle, Wash.
12. Curran, J., Driver, F., Ballard, J.W.O., and Milner, R.J. 1994. Phylogeny of *Metarhizium*: analysis of ribosomal DNA sequence data. Mycol. Res. **98**: 547–552.
13. Egger, K.N., and Sigler, L. 1993. Relatedness of the ericoid endophytes *Scytalidium vaccinii* and *Hymenoscyphus ericae* inferred from analysis of ribosomal DNA. Mycologia, **85**: 219–230.
14. Garber, R.C., Turgeon, B.G., Selker E.U., and Yoder, O.C. 1988. Organization of ribosomal RNA genes in the fungus *Cochliobolus heterostrophus*. Curr. Genet. **14**: 573–582.
15. Gorman, D., Xue, B., Hsiang, T., and Goodwin, P.H. 1994. Detection of *Leptosphaeria korrae* with the polymerase chain reaction and primers from the ribosomal internal transcribed spacers. Can. J. Bot. **72**: 342–346.
16. Hausner, G., Reid, J., and Klassen, G.R. 1993. On the subdivision of *Ceratocystis s. l.*, based on partial ribosomal DNA sequences. Can. J. Bot. **71**: 52–63.
17. Kohn, L.M. 1992. Developing new characters for fungal systematics: an experimental approach for determining the rank of resolution. Mycologia, **84**: 139–153.
18. Kurtzman, C.P. 1993. Systematics of the ascomycetous yeasts assessed from ribosomal RNA sequence divergence. Antonie Leeuwenhoek, **63**: 165–174.
19. Lewin, R. 1992. Complexity: life at the edge of chaos. Collier Books, Macmillan Publishing Company, New York.
20. Lewontin, R.C. 1991. Biology as ideology: the doctrine of DNA. Anansi Press, Concord, Ont.
21. LoBuglio, K.F., Pitt, J.I., and Taylor, J.W. 1993. Phylogenetic analysis of two ribosomal DNA regions indicates multiple independent losses of a sexual *Talaromyces* state among asexual *Penicillium* species in subgenus *Biverticillium*. Mycologia, **85**: 592–604.
22. LoBuglio, K.F., Pitt, J.I., and Taylor, J.W. 1994. Independent origins of the synnematous *Penicillium* species, *P. duclauxii*, *P. clavigergum*, and *P. vulpinum*, as assessed by two ribosomal DNA regions. Mycol. Res. **98**: 250–256.
23. Logrieco, A., Peterson, S.W., and Wicklow, D.T. 1990. Ribosomal RNA comparisons among taxa of the terverticillate Penicillia. *In* Modern concepts in *Penicillium* and *Aspergillus* classification. *Edited by* R.A. Samson and J.I. Pitt. Plenum Press, New York. pp. 343–355.
24. Logrieco, A., Peterson, S.W., and Bottalico, A. 1991. Phylogenetic affinities of the species in *Fusarium* section *Sporotrichiella*. Exp. Mycol. **15**: 174–179.
25. Luttrell, E.S. 1977. Correlations between conidial and ascigerous state characters in *Pyrenophora*, *Cochliobolus* and *Setosphaeria*. Rev. Mycol. **41**: 271–279.
26. Martin, F.N. 1990. Variation in the ribosomal DNA repeat unit within single-oospore isolates of the genus *Pythium*. Genome, **33**: 585–591.
27. Mayr, E. 1988. Towards a new philosophy of biology. Harvard University Press, Cambridge, Mass.
28. Moukhamedov, R., Hu, X., Nazar, R.N., and Robb, J. 1994. Use of polymerase chain reaction amplified ribosomal intragenic sequences for the diagnosis of *Verticillium tricorpus*. Phytopathology, **84**: 256–259.
29. Neuvéglise, C., Brygoo, Y., Vercambre, B., and Riba, G.

1994. Comparative analysis of molecular and biological characteristics of strains of *Beauveria brongniartii* isolated from insects. Mycol. Res. **98**: 322–328.
30. O'Donnell, K. 1992. Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). Curr. Genet. **22**: 213–220.
31. Perkins, D.D. 1991. In praise of diversity. *In* More gene manipulations in fungi. *Edited by* J.W. Bennet and L.L. Lasure. Academic Press, New York. pp. 3–26.
32. Peterson, S.W. 1993. Molecular genetic assessment of relatedness of *Penicillium* subgenus *Penicillium*. *In* The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. *Edited by* D.R. Reyolds and J.W. Taylor. C.A.B. International, Wallingford, U.K. pp. 121–128.
33. Peterson, S.W., and Logrieco, A. 1991. Ribosomal RNA sequence variation among interfertile strains of some *Gibberella* species. Mycologia, **83**: 397–402.
34. Prusinkiewicz, P., and Lindenmayer, A. 1990. The algorithmic beauty of plants. Springer-Verlag, New York.
35. Reynolds, D.R., and Taylor, J.W. 1991. Nucleic acids and nomenclature: name stability under Article 59. *In* Improving the stability of names: needs and options. *Edited by* D.L. Hawksworth. Regnum Veg. **123**: 171–177.
36. Reynolds, D.R., and Taylor, J.W. (*Editors*). 1993. The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. International Mycological Institute, C.A.B. International, Wallingford, U.K.
37. Roberts, A.N., and Yanofsky, C. 1989. Conidiation-specific genes of *Neurospora crassa*. Nucleic Acids Res. **17**: 197–214.
38. Saenz, G.S., Taylor, J.W., and Gargas, A. 1994. 18S rRNA gene sequences and supraordinal classification of the Erysiphales. Mycologia, **86**: 212–216.
39. Schwarz, M.B. 1922. Das zweigensterben der olmaen trauerweiden und pfirschbaume. Med. Phytopathol. Lab. Willie Commelin Scolten **5**: 1–73.
40. Seifert, K.A. 1993. Integrating anamorphic fungi into the fungal system. *In* The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. *Edited by* D.R. Reyolds and J.W. Taylor. C.A.B. International, Wallingford, U.K. pp. 79–85.
41. Sherriff, C., Whelan, M.J., Arnold, G.M., Lafay, J.-F., Brygoo, Y., and Bailey, J.A. 1994. Ribosomal DNA sequence analysis reveals new species groupings in the genus *Colletotrichum*. Exp. Mycol. **18**: 121–138.
42. Spatafora, J.W., and Blackwell, M. 1994. The polyphyletic origins of ophiostomatoid fungi. Mycol. Res. **98**: 1–9.
43. Sreenivasaprasad, S., Brown, A.E., and Mills, P.R. 1993. Coffee berry disease pathogen in Africa: genetic structure and relationship to the group species *Colletotrichum gloeosporioides*. Mycol. Res. **97**: 995–1000.
44. Sreenivasaprasad, S., Mills, P.R., and Brown, A.E. 1994. Nucleotide sequence of the rDNA spacer 1 enables identification of isolates of *Colletotrichum* as *C. acutatum*. Mycol. Res. **98**: 186–188.
45. Tasi, H.-F., Liu, J.-H., Staben, C., Christensen, M.J., Latch, G.C.M., Siegel, M.R., and Schardl, C.L. 1994. Evolutionary diversification of fungal endophytes of tall fescue grass by hybridization with *Epichloë* species. Proc. Natl. Acad. Sci. U.S.A. **91**: 2542–2546.
46. Taylor, J.W., Bruns, T.D., and White, T.J. 1990. Can the amount of molecular divergence define species and genera: comparison of Sordariales and Agaricales. Fourth International Mycological Congress, Regensburg, Germany. Abstr. 1G-343/3.
47. Timberlake, W.E. 1990. Molecular genetics of *Aspergillus* development. Annu. Rev. Genet. **24**: 5–36.
48. Viljoen, C.D., Wingfield, B.D., and Wingfield, M.J. 1993. Comparison of *Seiridium* isolates associated with cypress canker using sequence data. Exp. Mycol. **17**: 323–328.
49. Waldrop, M.M. 1992. Complexity: the emerging science at the edge of order and chaos. Simon & Shuster, New York.
50. Wegner, T., Tyler, B., Peterson, M., and Brandenhorst, P. 1992. Fractals for Windows. Waite Group Press, Corte Madera, Calif.
51. Wiley, E.O., Siegel-Causey, D., Brooks, D.R., and Funk, V.A. 1991. The compleat cladist. A primer of phylogenetic procedures. Univ. Kans. Mus. Nat. Hist. Spec. Publ. **19**: 1–158.
52. Williams, D.M., and Round, R.E. 1994. Systematics and science: the role of authority. Nova Hedwigia, **59**: 219–223.