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Modelling and visualizing morphology in the fungus *Alternaria*

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ABSTRACT

Alternaria is one of the most cosmopolitan fungal genera encountered and impacts humans and human activities in areas of material degradation, phytopathology, food toxicology, and respiratory disease. Contemporary methods of taxon identification rely on assessments of morphology related to sporulation, which are critical for accurate diagnostics. However, the morphology of *Alternaria* is quite complex, and precise characterization can be laborious, time-consuming, and often restricted to experts in this field. To make morphology characterization easier and more broadly accessible, a generalized statistical model was developed for the three-dimensional geometric structure of the sporulation apparatus. The model is inspired by the widely used grammar-based models for plants, Lindenmayer-systems, which build structure by repeated application of rules for growth. Adjusting the parameters of the underlying probability distributions yields variations in the morphology, and thus the approach provides an excellent tool for exploring the morphology of *Alternaria* under different assumptions, as well as understanding how it is largely the consequence of local rules for growth. Further, different choices of parameters lead to different model groups, which can then be visually compared to published descriptions or microscopy images to validate parameters for species-specific models. The approach supports automated analysis, as the models can be fit to image data using statistical inference, and the explicit representation of the geometry allows the accurate computation of any morphological quantity. Furthermore, because the model can encode the statistical variation of geometric parameters for different species, it will allow automated species identification from microscopy images using statistical inference. In summary, the approach supports visualization of morphology, automated quantification of phenotype structure, and identification based on form.

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Introduction

The genus *Alternaria* represents some of the most common fungi encountered world-wide. They have been recovered in almost every ecosystem in soil and in association with organic debris of all types and are ubiquitous agents of decay (Rotem 1994). Many species pathogenic to plants and are listed as one of the top ten phytopathogens in terms of the number of recorded hosts (Farr et al. 1989). Some species have also been recorded as opportunistic pathogens of humans, particularly in immunocompromised patients (de Hoog et al. 2000). *Alternaria* are easily dispersed via windborne conidia and are among the most common potent airborne allergens (Wilken-Jensen & Gravesen 1984). Thus, the study of *Alternaria* impacts many disciplines and the correct identification of species is critical in terms of management and mitigation of its effects.

Alternaria diagnostics, as with most fungi, is primarily based on morphological characteristics of the reproductive structures. These structures can be quite complex and encompass considerable diversity even between closely related taxa. Moreover, sporulation structures exhibit considerable plasticity depending on environmental parameters making identification of species difficult if reproductive structures are generated under different conditions of growth. Among *Alternaria* species, small-spored catenulate taxa in the alternata species-group represent the most challenging in terms of accurate diagnostics due to their complex microscopic three-dimensional (3D) sporulation apparatus (Fig 1). The process of taxon identification currently requires observation of isolates at both low and high magnification and quantification of structures from microscopic images under standardized culture conditions. This may require considerable time, recording various morphological parameters such as length of primary conidiophore, degree of catenation, branching patterns, branching angles, spore shapes, and surface ornamentation. This method of classification is often subjective, time-consuming, and prone to error. Mathematical models that can represent fungal structures quantitatively and enable statistical analysis of these data automatically would be a tremendous asset for confirmation of taxon identity and for classification of unknown samples. Developing a mathematical representation of the geometric form for *Alternaria* for quantification of morphology would also assist linking morphometrics to biological and ecological function.

Previous efforts towards modelling morphology of filamentous fungi have focused on the 2D form of colonies of single specimens. Early work (Tunbridge & Jones 1995) used a parametric Lindenmayer-system model (Lindenmayer 1968, 1975) to simulate growth of 2D colonies of the fungus *Aspergillus nidulans*, using biological understanding of the flow of nutrients through the fungus. Other works have used differential equations to explain pattern formation in fungal colony growth (Lopez & Jensen 2002; Grimm et al. 2005; Moore et al. 2005). For example, Lopez & Jensen (2002) developed a 2D stochastic model of mycelium morphology generated during the growth of *Aspergillus oryzae* on solid agar by taking into account the effects of accumulation of toxic metabolites and depletion of nutrients. Incorporating growth in three dimensions, Moore et al. (2005) created the neighbour-sensing model for colony growth

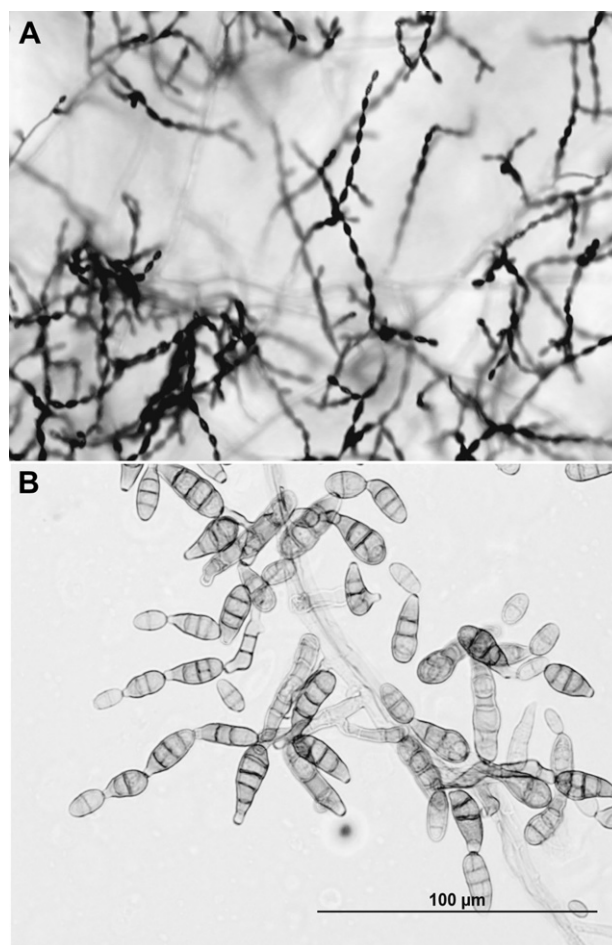


Fig 1 – A small-spored catenulate *Alternaria* species, *A. alternata*. (A) Image from a Petri dish culture using a stereo microscope under low magnification. (b) Image from a prepared slide mount using a compound microscope under high magnification. Note that accurate visualization of the 3D qualities of the sporulation apparatus is difficult using either method.

using lines as a simple representation for hyphae and spores. A number of adjustable kinetics parameters provide sufficient power to create a variety of colony patterns which the authors link to observed patterns from three genera – *Boletus*, *Amanita*, *Tricholoma*. The models use lines and disks to represent the hyphae and spores and can be visualized in 3D space. Grimm et al. (2005) reviewed 3D models where morphology is determined from observations of microscopy images using only total hyphal length and the number of growing tips, as well as models of fungal growth incorporating vesicle kinematics. In summary, these models can work well when the goal is to generate the overall shape and structure of a fungal colony. However, to acquire quantitative information about the 3D morphological structures, a model representing the geometry at the organ level is needed.

Lindenmayer-systems (L-systems) are one type of model suitable for this task. They are grammar-based models invented by the biologist A. Lindenmayer as a mathematical tool to model cellular interactions in plants (Lindenmayer

1968, 1975). Specifically, these systems were introduced to model morphogenetic processes in growing multicellular organisms (Frijters & Lindenmayer 1974) and subsequently have been used as a realistic model of growth for many plant species (Prusinkiewicz *et al.* 1988). L-systems can give an approximation to the biological structure by simultaneously replacing all states of a growing structure with new states according to rules for biological growth (Frijters & Lindenmayer 1974). L-systems model plants as a set of connected self-similar substructures whose assembly can be described by a formal set of rules for growth. For example, using capital letters to represent plant substructures, an L-system that takes the initial string A and the rule 'replace A with ABA at each iteration,' will produce ABA after the first application of the rule, ABABABA after the second application, and so on. In the context of modelling fungal growth such as that which might occur during *Alternaria* sporulation, an example rule for growth would be to generate an apical hypha after an existing spore on some conidiophore branch. Simultaneously, the model can generate a new spore after another existing spore on another branch (see Fig 2).

Formally, an L-system is a tuple $\langle V, \sigma, \omega, P, \pi \rangle$, where V is the set of symbols that can be replaced at each iteration (the alphabet), σ is a set of formal parameters, ω is the starting string (axiom), P is a set of production rules, and π is a probability distribution over the rules. Furthermore, because plant growth exhibits random variation within the constraints of the rules for growth, the L-system rules can be stochastically applied to obtain a more realistic model. L-systems have been successfully used for real time design, animation and rendering of plants and trees (Nishida 1980; Smith 1984; Oppenheimer 1986; Weber & Penn 1995), modelling the evolution of inflorescences in plants (Prusinkiewicz *et al.* 2007), modelling and rendering plant ecosystems (Deussen *et al.* 1998), and more specifically, for modelling peach trees (Allen *et al.* 2005), *Fraxinus pennsylvanica* shoots (Hammel *et al.* 1995), proteins (Escuela *et al.* 2005), and herbaceous plants (Prusinkiewicz *et al.* 1988). Other studies have used stochastic parametric L-systems, where one symbol can be replaced with one of several options, governed by a probability (Prusinkiewicz *et al.* 1988). For example, the symbol 'branch'

can be replaced with a 'branch and a leaf' 60 % of the time, and with a 'branch and a bud' 40 % of the time.

When used to model plants, the symbols of the L-system usually represent branches, leaves, and flowers. Inspection reveals that the key elements that make L-systems applicable to plants also apply to the sporulation structure of *Alternaria*, which is also assembled from a small set of components. Of course, instead of branches, leaves, and flowers, the terminal symbols represent *Alternaria* components such as hyphae and spores. Further, much of the structure is recursive in that a branch attached to a given structure can consist of a structure similar to the structure it is attached to. Hence, it is natural to use L-system framework for a stochastic geometric model for the morphology of *Alternaria*, and the immediate goal of this work was to explore this idea. A parallel objective was to ensure that the models could be fit to image data, thereby providing a path towards automated quantification of form with respect to the geometric quantities and relations at the organ level. More broadly, the goal is to develop a sufficiently flexible system that is applicable to many other fungal groups defined by their sporulation structures, which would support comprehensive morphometric analyses of fungi in general.

Materials and methods

Taxa used in this study

Four species of *Alternaria* were initially selected as templates for model development: *Alternaria alternata*, *Alternaria tenuissima*, *Alternaria gaisen*, and *Alternaria arborescens*. These species are all in the taxonomically challenging alternata species-group and were selected because they represent the spectrum of morphological structures most commonly encountered among small-spored, catenulate *Alternaria* species. Prior morphological data for each of these taxa were obtained primarily from the extensive descriptions provided in the work of Simmons (2007), and complemented by work of others (Pryor & Michailides 2002; Hong *et al.* 2006).

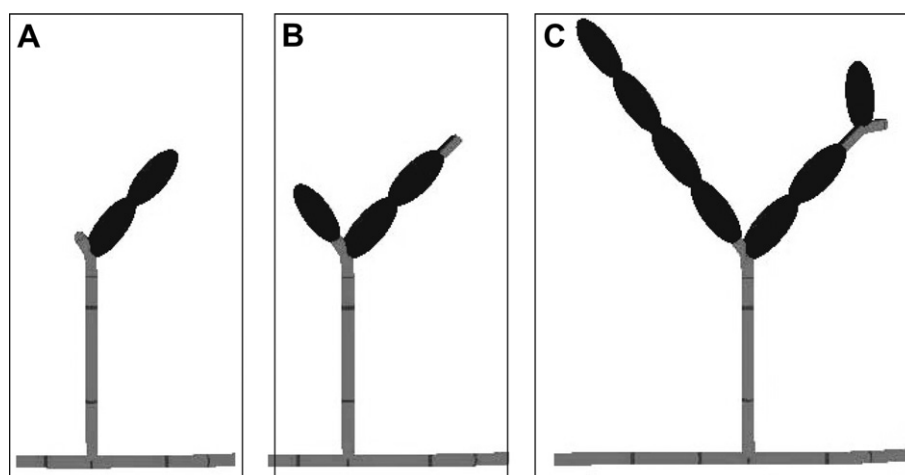


Fig 2 – Example of applying L-system rules for three recursion steps in the context of modelling fungi. The model simultaneously creates an apical development after a spore, and continues to grow a chain with an additional spore.

Development of the *Alternaria* L-system

To summarize the morphological features considered in this work, *Alternaria alternata* consists of single sub-erect conidiophores that are relatively short, usually simple, or 1–3 branched (Fig 3). Borne on this primary structure are branching chains of small conidia, which are catenated to varying degrees (5–15 conidia in length). Branching within these conidial chains can take one of two forms: the elongation of the primary or secondary conidiophores following terminal conidium formation (sub-conidium conidiophore elongation; SCE) or through the development of a lateral secondary conidiophore emerging from one of the conidium cells (lateral intra-conidium conidiophore development; LICD; Fig 3). This type of branching may produce numerous secondary, tertiary, and even quaternary branching, resulting in a quite complex sporulation structure. In addition, secondary conidiophores development can occur at the apical terminus of a conidium (apical terminus conidiophore development; ATCD), and the length of this development may be a short single cell or extended to varying lengths before a subsequent conidium is formed.

Alternaria tenuissima has similar branching dynamics but the degree of branching is much reduced (Pryor & Michailides 2002; Simmons 2007). This species has a pattern of moderate to long chains of 5–15 or more of long-narrow conidia, with one or few LICD and a similar number of ATCD and SCE (Fig 4A). *Alternaria gaisen* has solitary and uncrowded simple primary conidiophores. Conidial chains are usually unbranched, or with

abbreviated LICD, with 3–5 conidia in catenation (Fig 4B). Each conidium except the terminal one usually has an ATCD of one cell (Simmons 2007).

Alternaria arborescens is somewhat distinct from the previous species in having a long, well-defined primary conidiophore with a few sub-terminal branches. Primary conidiophores produce a terminal cluster of branches and short, but highly branched conidial chains (Simmons 2007). Branching occurs almost exclusively by SCE resulting in numerous secondary, tertiary, and quaternary conidial chains, and branching via LICD is very rare. The occurrence of ATCDs are common and of variable lengths (Fig 4C).

In general, to identify these species of *Alternaria*, mycologists use the degree of branching generated by the three different types of developments after a spore is created (percent of primary conidiophore with SCE, LICD, or ATCD), the number of these developments per positive conidiophores, and the length of spore chains. There are a limited number of possibilities for the structures the fungus can develop during its growth. Each branch in a primary conidiophore development is capable of producing spores. The hyphal cells in a branch can develop another hyphal cell through apical growth or form a spore at its tip. After a spore develops, several structures can occur, depending on the species: 1) another spore, 2) a lateral secondary conidiophore coming from one cell of the spore (LICD), 3) an apical secondary conidiophore coming from the tip (ATCD), or 4) SCE occurring in the hypha cell immediately below the spore. The possibilities for further development repeat,

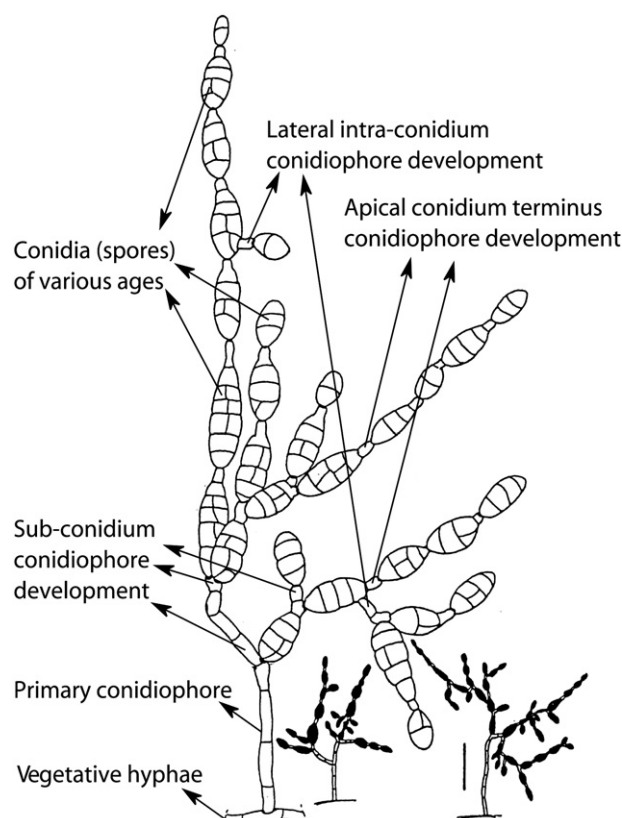


Fig 3 – *Alternaria alternata*: portions of sporulation clumps and habits from PCA (left) and Hay (right). The figure identifies the various spore and conidiophore developments. EGS 34-016. Bars ~ 50 μ m. Line drawings copyright by E.G. Simmons, 1999. Mycotaxon 70:336.

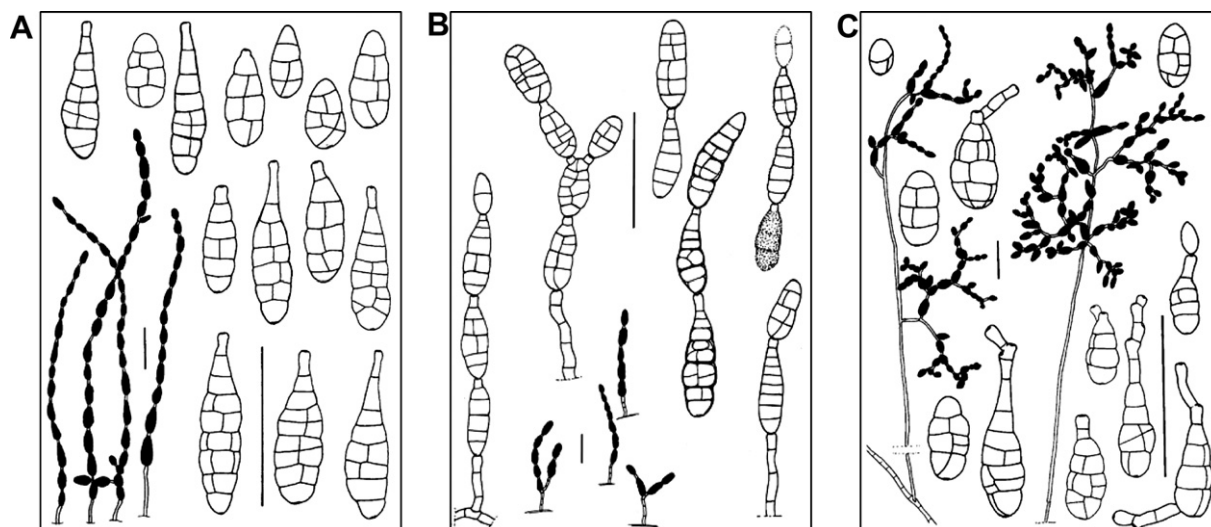


Fig 4 – (A) *Alternaria tenuissima*: portions of sporulation clump. Line drawings copyright by E.G. Simmons, 1993. Mycotaxon 48:124. (B) *Alternaria gaisen*: portions of sporulation clump. Line drawings copyright by E.G. Simmons, 1999. Mycotaxon 70:342. (C) *Alternaria arborescens*: portions of sporulation clump. Line drawings copyright by E.G. Simmons, 1993. Mycotaxon 48:121.

thus defining a recursive growth pattern. This process of growth creates a self-similar structure, which was modelled with a parametric stochastic L-system.

To develop the L-systems, the rules and parameters for each species were initially estimated manually using published characteristics, and then these parameters were tuned using a user-friendly online modelling tool (Taralova 2008). The online tool contains options for all rules and parameters and it enables instant visualization of the 3D model in the user's browser after each change in the settings. The web tool sends the L-system rules to a C++ program, which specifies how the L-system model should be executed. After execution of the program, the output of the L-system model is rendered into a 3D structure generated using VRML (Virtual Reality Markup Language) for ease of visualization.

Results

Development of the *Alternaria* L-system

The rules for growth described above define the basis of the L-system model. The L-system that was developed incorporated 34 rules, which encompassed the myriad of morphological characteristics that might be encountered when examining *Alternaria* species in the *alternata* species-group. To facilitate the development and analysis of the *Alternaria* generator, the online web tool was used to input the model parameters.

The L-system consists of a set of morphological structures (spores, hyphal cells, etc.), each with independent stochastic parameters (length, angles, etc.), a set of rules for growth, each associated with a probability distribution. The model also includes parameters for the probability distributions used in the development of the morphological structures and the replacement rules. The main rules in the model describe the growth of the vegetative hypha, the conidiophore branches, and the formation of the sporulation structures LICD, ATCD, and SCE. The

parameters for the probability distributions used in the rules were not available in prior descriptions of the species. Hence, they were estimated from data and incorporated in the model to generate natural-looking structures resembling published descriptions. The branching angles were also selected from a user-defined probability distribution with user-defined parameters, such as uniform, Gaussian, or gamma probability distribution. The complete set of parameters and rules is available for exploration through the online tool (Taralova 2008). The components of the L-system are defined as follows.

Each morphological structure is represented by a 3D geometric shape with a set of parameters. Each symbol π denotes a parameter drawn from some probability distribution, which is specified by the user, along with the distribution parameters (the available distributions are uniform, Gaussian, Gamma, and frequencies of occurrence).

The morphological structures for *Alternaria* are defined in L-system notation using the strings S , h , H , A , and L as follows:

$$S : \{ \pi_{\theta}, \pi_{\varphi}, s_{\text{age}}, \pi_{\text{sections}}, s_X^i(s_{\text{age}}), s_Y^i(s_{\text{age}}), s_Z^i(s_{\text{age}}), s_{\text{color}} \} \text{ (spore)}$$

where the parameters are: $\pi_{\theta}, \pi_{\varphi}$ – polar angles with respect to the previous structure; s_{age} – age of the spore, automatically updated at each recursion step; π_{sections} – number of ellipsoidal sections which make up the spore; $s_X^i(s_{\text{age}}), s_Y^i(s_{\text{age}}), s_Z^i(s_{\text{age}})$ – scale for each ellipsoidal section in the X, Y, and Z axis by an amount which is a function of the current age of the spore;

$$h : \{ \pi_{\text{length}}, \pi_{\theta}, \pi_{\varphi} \} \text{ (hyphal cell)}$$

with parameters: $\pi_{\theta}, \pi_{\varphi}$ – polar angles with respect to the previous structure; π_{length} – length of the hypha cell drawn from a probability distribution;

$$H : \{ \pi_{\text{number}}, h \} \text{ (sequence of hyphal cells)}$$

where π_{number} is the number of hypha cells to create and h has parameters defined above;

$$A : \{ \pi_{\text{length}}, \pi_{\theta}, \pi_{\varphi}, t_{\text{age}} \} \text{ (one apical hyphal cell)}$$

with same set of parameters as a hypha cell, with the addition of t_{age} , denoting the age of the apical cell;

$L : \{ \pi_{length}, \pi_{\theta}, \pi_{\varphi}, t_{age} \}$ (one lateral hyphal cell)

with the same set of parameters as an apical cell.

To make the model flexible and amenable to fine-grain tuning, the parameters π_{length} , π_{θ} , π_{φ} , π_{number} , t_{age} , from the above morphological structures were made specific to each structure. This allows the user to specify independent probability distributions for each parameter of the different morphological structures. For instance, the hypha cell length for an apical development can be drawn from a Gaussian distribution with mean 10 and variance 2, while for a lateral development it can be drawn from a Gaussian with mean 6 and variance 1.

The L-system rules for growth of *Alternaria* species are defined as follows. The vegetative hyphae produce primary conidiophore branches, which are separated from each other by a number of vegetative hyphal cells of different lengths and relative orientation in three dimensions (Fig 5). This rule for growth was represented in L-system notation by the following production:

$$V \xrightarrow{\pi_1} H[B]V \xrightarrow{\pi_2} V \xrightarrow{\pi_3} \epsilon, \text{ (vegetative hypha)}$$

where V denotes vegetative hypha that is replaced with one of three possibilities with probabilities π_1 , π_2 , and π_3 , where it is always the case that $\sum \pi_i = 1$. Specifically, with probability π_1 , V is replaced by a sequence of hyphal cells (denoted by H), a primary conidiophore branch (B), and the original vegetative

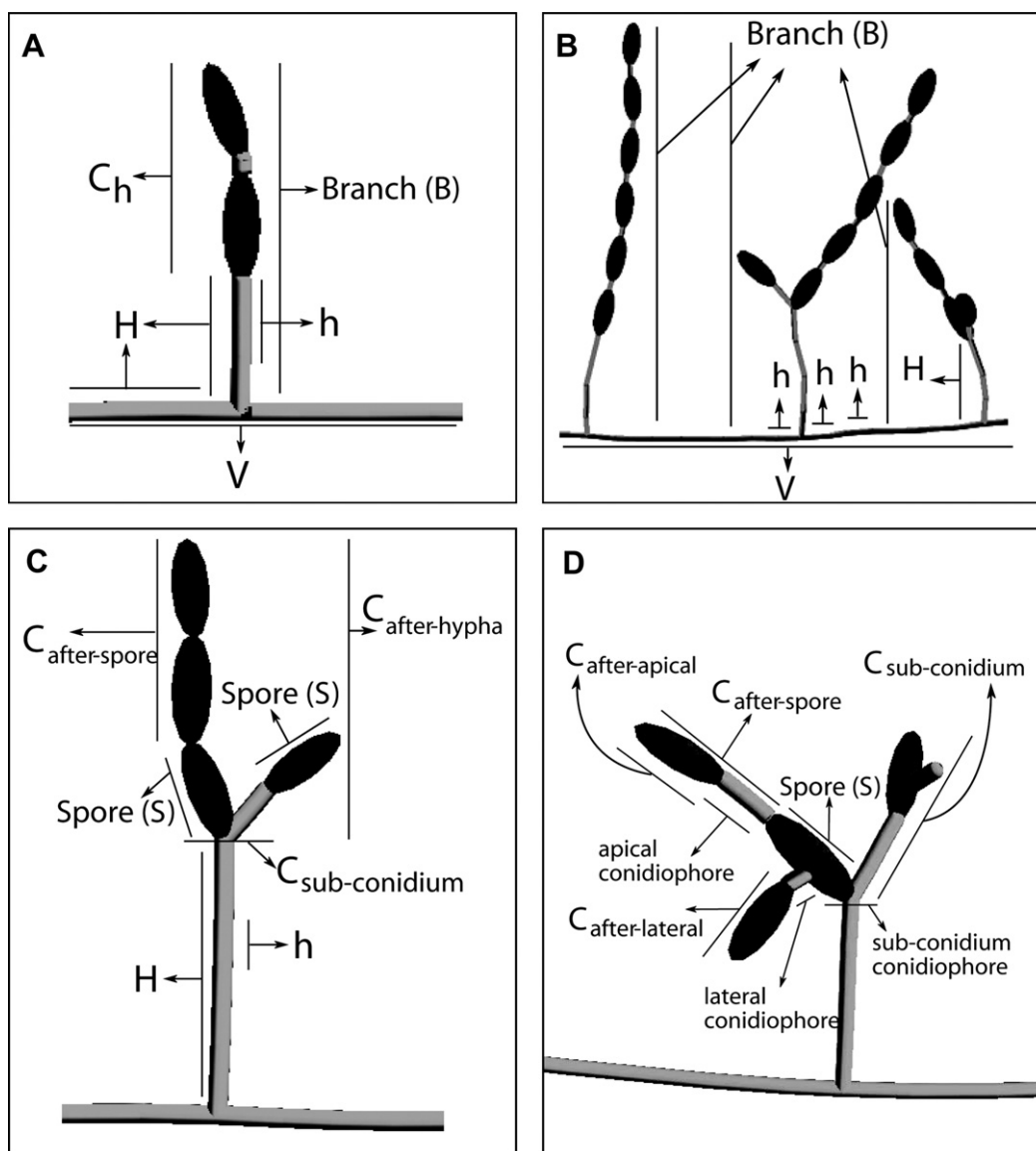


Fig 5 – (A) Example of the L-system rule for vegetative hypha growth. The vegetative hypha (V) produces primary conidiophore branches (B), which are separated by a number of hyphal cells (h) of different lengths and relative orientation in three dimensions. **(B)** Example of the vegetative hypha rule for growth applied 12 times. The image shows the first three branches of the resulting structure. **(C)** Example of the possible structures that can develop after a primary conidiophore hypha cell has been created, using the L-system rules for *A. alternata*. **(D)** An illustration of the L-system rule for generating sub-conidium elongations, and apical and lateral conidiophore developments.

hypha itself, V , thereby lengthening the hypha and inserting a branch (Fig 5A). The symbols $[$ and $]$ represent the start and end of a branch. With probability π_2 this rule will skip a step, i.e., it will not produce any structures and will be replaced by V to be evaluated again in the next recursion step. The last possibility, occurring with frequency π_3 , is to cease producing any new structures in the future, i.e., to terminate the rule replacement, which is indicated by the symbol ϵ . Fig 5B shows an example of several applications of this rule.

A primary conidiophore branch grows by creating hyphal cells of different lengths and at different relative orientations, and it is capable of producing various conidiophore developments. In L-system notation, the replacement rule for a primary conidiophore hypha cell B is given by

$$B \xrightarrow{\pi_1} HC_{\text{after-hypha}} \xrightarrow{\pi_2} B \xrightarrow{\pi_3} \epsilon, \text{ (primary conidiophore)}$$

where H generates a sequence of hyphal cells (i.e., elongation of the primary conidiophore) and $C_{\text{after-hypha}}$ represents a set of possible structures that can develop after a primary conidiophore hypha cell has been created. One such structure is a sub-conidium conidiophore, represented by $C_{\text{sub-conidium}}(t_{\text{sc}})$, which is followed by a spore, S . A second such structure is simply a spore, S . The next possible action after developing a primary conidiophore cell is to skip a step and not develop anything during this iteration, but to resume growth at the next iteration, which is represented by replacement with $C_{\text{after-hypha}}$ without creating any other structures. The next possible conidiophore developments after a spore has been formed are denoted by $C_{\text{after-spore}}(t_{\text{as}})$. The L-system representation for the replacement of $C_{\text{after-hypha}}$ is

$$\begin{aligned} C_{\text{after-hypha}} &\xrightarrow{\pi_1} [C_{\text{sub-conidium}}(t_{\text{sc}})]SC_{\text{after-spore}}(t_{\text{as}}) \\ &\xrightarrow{\pi_2} SC_{\text{after-spore}}(t_{\text{as}}) \\ &\xrightarrow{\pi_3} C_{\text{after-hypha}} \\ &\xrightarrow{\pi_4} \epsilon, \text{ (conidiophore options after a hypha)} \end{aligned}$$

where each π_i is the probability of choosing each replacement option, and $\sum \pi_i = 1$. Fig 5C shows an example of this rule. Furthermore, some of the conidiophore developments can be constrained to occur only after a spore reaches a certain 'age,' as defined by the number of iterations elapsed since the spore's creation. For example, $C_{\text{sub-conidium}}(t_{\text{sc}})$ enforces that a SCE will develop only after the preceding spore has reached age t_{sc} , where this parameter was drawn from a user-specified probability distribution.

The sub-conidium conidiophore development, $C_{\text{sub-conidium}}$, can continue to grow hyphal cells of different lengths and orientations, or it can be followed by a spore, or future execution can be terminated. The L-system rule is

$$\begin{aligned} C_{\text{sub-conidium}} &\xrightarrow{\pi_1} hC_{\text{sub-conidium}} \\ &\xrightarrow{\pi_2} SC_{\text{after-spore}}(t_{\text{as}}) \xrightarrow{\pi_3} \epsilon, \\ &\text{(conidiophore options following a sub - conidium)} \end{aligned}$$

Fig 5D shows an illustration of this rule.

After a spore has been developed, there are several possibilities: create another spore, or an apical conidiophore

development (A), or a LICD (L), skip this iteration, or terminate. The creation of one of these developments will determine the structures that can be generated in the next step. This is represented by the L-system rule:

$$\begin{aligned} C_{\text{after-spore}} &\xrightarrow{\pi_1} SC_{\text{after-spore}} \xrightarrow{\pi_2} AC_{\text{after-apical}}(t_{\text{aa}}) \\ &\xrightarrow{\pi_3} LC_{\text{after-lateral}}(t_{\text{al}}) \xrightarrow{\pi_4} [C_{\text{sub-conidium}}(t_{\text{sc}})]SC_{\text{after-spore}}(t_{\text{as}}) \\ &\xrightarrow{\pi_5} \epsilon, \text{ (conidiophore options after a spore)} \end{aligned}$$

where creating a spore S is recursively followed by $C_{\text{after-spore}}$, creating an apical hypha A is followed by a set of possible developments denoted by $C_{\text{after-apical}}(t_{\text{aa}})$, and similarly a lateral hypha L is followed by $C_{\text{after-lateral}}(t_{\text{al}})$. The L-system rules used to describe the after-apical and after-lateral options are

$$\begin{aligned} C_{\text{after-apical}} &\xrightarrow{\pi_1} hC_{\text{after-apical}} \xrightarrow{\pi_2} SC_{\text{after-spore}}(t_{\text{as}}) \xrightarrow{\pi_3} C_{\text{after-apical}} \\ &\xrightarrow{\pi_4} \epsilon, \text{ (conidiophore options after apical hypha)} \end{aligned}$$

$$\begin{aligned} C_{\text{after-lateral}} &\xrightarrow{\pi_1} hC_{\text{after-lateral}} \xrightarrow{\pi_2} SC_{\text{after-spore}}(t_{\text{as}}) \xrightarrow{\pi_3} C_{\text{after-lateral}} \\ &\xrightarrow{\pi_4} \epsilon, \text{ (conidiophore options after lateral hypha)} \end{aligned}$$

where h will create another apical hyphal cell, followed again by the possible after-apical developments $C_{\text{after-apical}}$, or create a spore S , skip this turn, or terminate, and similarly for the lateral development rule. Fig 5D shows an example of applying the above three rules.

As observed from data, a spore can generate a lateral intra-conidium or an apical conidium development only after it matures. In the L-system model the maturity of a structure is defined by its age, which starts at zero when the structure is created and increases by one at each iteration. The age at which a structure matures is denoted in parenthesis by the user-defined parameter t . For example, the structure $C_{\text{after-spore}}(t_{\text{as}})$ will not be substituted according to its replacement rule until the age requirement is met. Once the condition is met, the L-system replacement rule will be executed and the parameter t_{as} can then be ignored (thus t_{as} does not occur on the left-hand side of any replacement rule). Furthermore, the age parameter is used by the $C++$ application to create a 3D representation of a spore with a size proportional to its age. In addition to an age parameter, the model includes parameters, which control the number of recursion steps, the random number generator seed used to draw values from the probability distributions, the length of a spore branch and the number of spores per branch.

The L-system model is able to generate different model instances using the same grammar and associated species parameters by changing the random number generator seed. Fig 6 shows three instances of individual conidiophores generated by the L-system rules with parameters developed for modelling *Alternaria alternata*. Fig 7 shows models of fungi allowed to grow extensively into a colony.

To verify that chosen parameters and the L-system rules can in fact generate fungi that visually appear similar to a variety of species, we used the web tool to generate numerous models that robustly create *A. alternata*, *Alternaria gaisen*, *Alternaria tenuissima*, and *Alternaria arborescens*. The models were visually examined and compared to data to confirm that they represent reasonable instances of each taxon. To facilitate this process, the 3D L-system models can be displayed in a virtual



Fig 6 – The model parameters designed for modelling *A. alternata* were used here to generate three different instances of this species using three different random seeds.

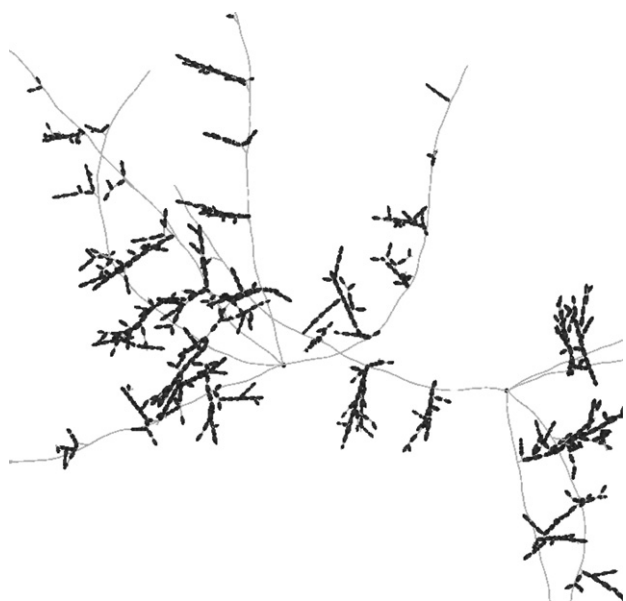


Fig 7 – Example of several strands of fungi produced by the L-system rules for *A. alternata* allowed to grow into a colony. This example simulates fungi growth on a Petri dish.

environment room where scientists can explore them in much greater detail and on a large scale. This is useful because it will enable the data and the model to be displayed together, the model fits verified, and the parameters that did not produce a good match can be corrected. For a comparison of the generated models with the drawings by E.G. Simmons see Fig 8.

Evaluation of model parameters

Two types of validations of the model parameters were performed, and a third method is proposed in the Discussion section as future work. Following development of a generalized form for the input of the various model parameters, a specific parameter-defined model was developed for each taxon. First, several instances of one species are generated and the models are visually examined for any flaws, comparing them to the existing descriptions of the species and to microscopy images. The model parameters are modified to better fit the developmental process of the species. These steps are repeated until new instances are visually similar to real data. L-system models are difficult to validate mathematically. However, since they incorporate the mechanisms for growth and morphology, they lend themselves to easy visual evaluation. This process of validation has been used in prior work that models biological structures (Fracchia & Ashton 1995; Hammel et al. 1995).

In addition to visual examination, a comparison of the models for each taxon in terms of the morphometrics that could be extracted was performed. For this model validation, 1000 instances of each taxon were generated using different random seeds. Several morphometrics were gathered from each sporulation structure generated which included LICD (Fig 9A), ATCD (Fig 9B), SCE (Fig 9C), and spore counts (Fig 9D) and comparisons were made among models to see if the selected metrics could be used to differentiate the taxa in question. The figures show the collective distributions of the various morphometrics are distinct for the four species modelled, i.e., the four taxa could be differentiated by the combined selected metrics. Even though these statistics depend directly on the manually selected species-wide model parameters, the results confirm the models we develop generate similar instances of the same species, and different instances across species.

Discussion

Alternaria includes some of the most difficult fungi to accurately identify and characterize based upon morphological criteria due to the production of complex and pleomorphic sporulation structures. To help visualize the morphology of these fungi and assist in diagnostics and taxonomy, a grammar-based 3D model for growth of *Alternaria* was developed. The model is general enough to accommodate the various morphological characteristics of the hundreds of species in the genus. The model incorporated numerous morphological parameters and by changing their values, instances of each species of the genus can be generated. In addition to stochastic application of rules similar to prior models, this work incorporates probability distributions for each model parameter to capture variations such as length of cells, angles, number of

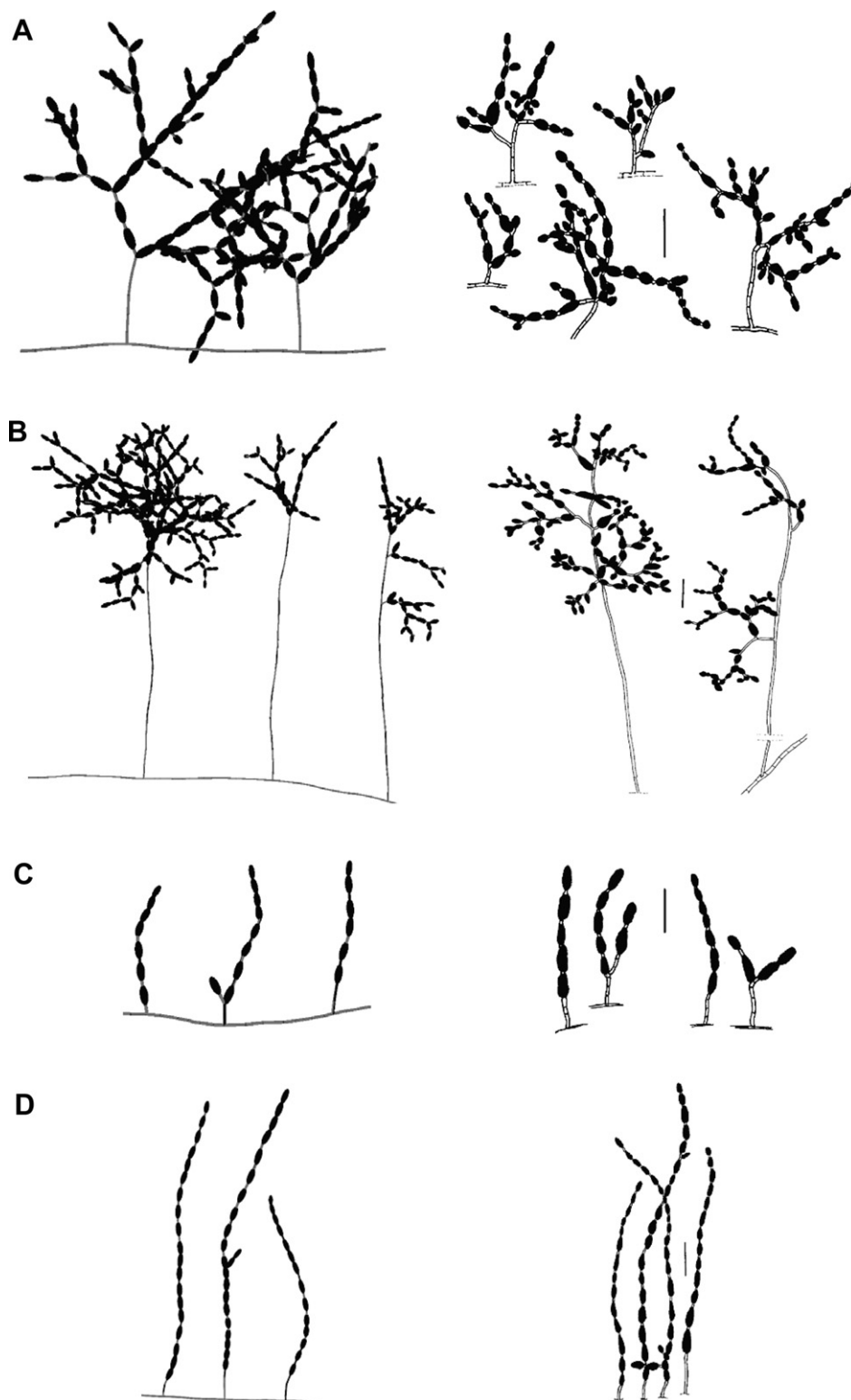


Fig 8 – (A) *A. alternata*, (B) *A. arborescens*, (C) *A. gaisen*, and (D) *A. tenuissima* generated with the modelling tool (left column) and compared to the hand drawings of Simmons (2007) (right column). The figure shows how the generated model instances are visually similar to the hand drawings of the various species. Line drawings copyright by E.G. Simmons (A) 1993. Mycotaxon 48:123, (B) 1993. Mycotaxon 48:121, (C) 1999. Mycotaxon 70:342, (D) 1993. Mycotaxon 48:124.

structures, etc. Furthermore, the developed model also allows time-dependent rules to be specified to better model developments, which occur only after a spore has matured (e.g., developing an LICD only after spore reaches a certain age).

Models can be conveniently experimented with using a publicly available web tool (Taralova 2008) that displays 3D model instances interactively using VRML. Further, the models are well suited for display in virtual reality environments.

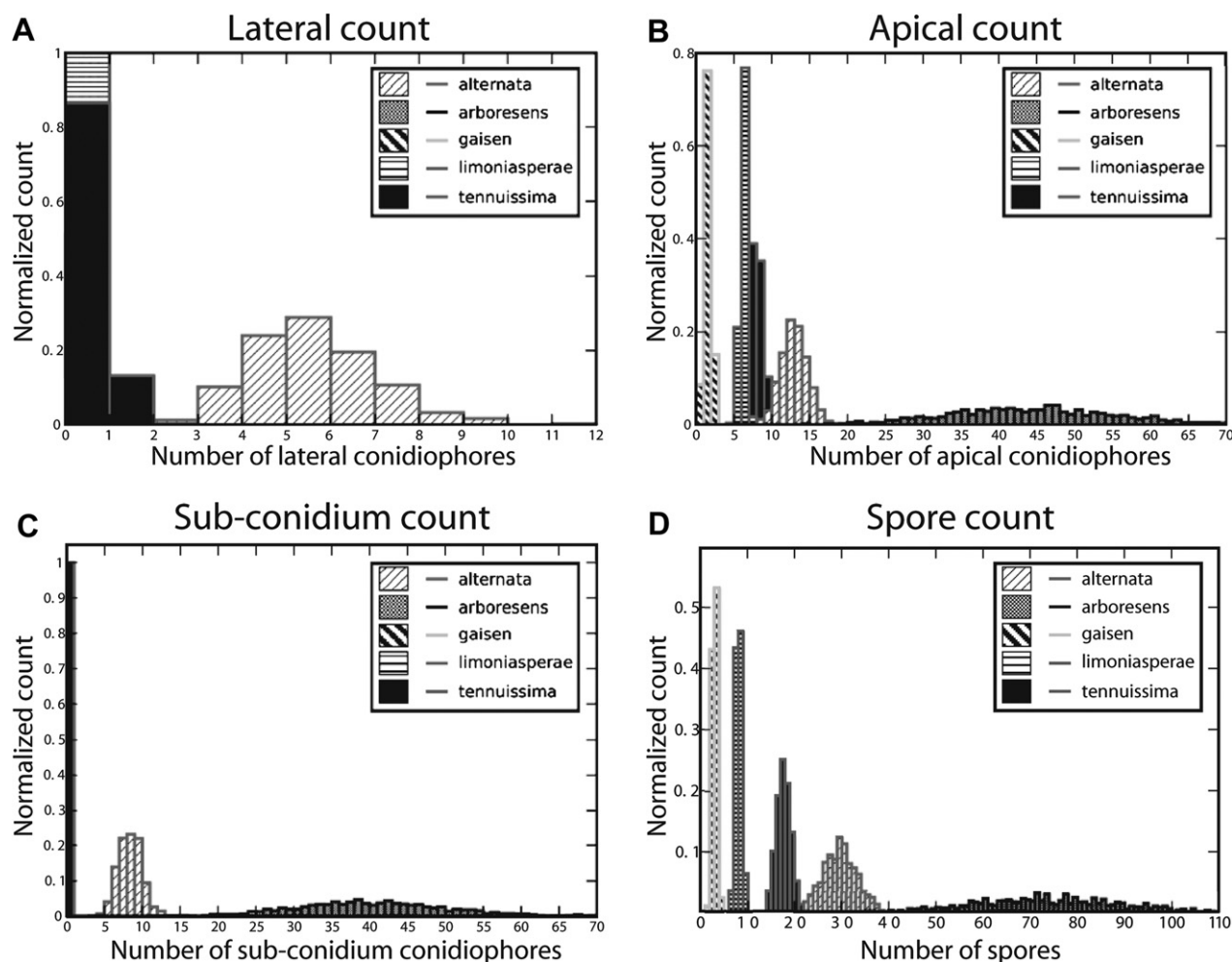


Fig 9 – (A) Normalized histograms of the count of lateral conidiophore structures obtained from 1000 instances of models of *alternata*, *arborescens*, *gaisen*, and *tenuissima*. The plot shows that the distribution of the lateral conidiophore structures is distinctive for some of the species. **(B)** Normalized histograms of the count of apical conidiophore structures obtained from 1000 instances of models of *alternata*, *arborescens*, *gaisen*, and *tenuissima*. The plot shows the four species can be distinguished using their apical conidiophore count. **(C)** Normalized histograms of the count of sub-conidium conidiophore structures obtained from 1000 instances of models of *alternata*, *arborescens*, *gaisen*, and *tenuissima*. The plot shows the sub-conidium count can be used to distinguish among some of the four species. **(D)** Normalized histograms of the count of spores obtained from 1000 instances of models of *alternata*, *arborescens*, *gaisen*, and *tenuissima*. The spore counts are distinctive across the four species.

The approach was explicitly designed to support fitting the mathematical model to microscopy images from unknown fungal samples with a computer program. In parallel work our group has developed a method to fit a simplified version of this model to image stacks using Bayesian inference (Schlecht et al. 2007). Adapting that work to the full model described here will enable automated analysis and classification of a wide range of *Alternaria* species. In addition, models extracted from image stacks can be overlaid on corresponding image data in virtual environments for detailed inspection and evaluation. Finally, fitting groups of images from the same species will allow automated improvement of the manually set parameters. These capabilities will be implemented in future work.

This approach will assist taxonomy in two ways. Because the model encodes what is known about the statistics of the form of *Alternaria*, fitting it to image data may be more robust

and reliable than manual identifications, especially if such identifications are performed by non-specialists. Second, because the model represents the form through the arrangement of meaningful sub-components, any reasonable morphometric computation is straightforward, which is distinctly different from developing ‘one-off’ solutions for measurements of interest for a given experiment.

Learning the structure of an object is one of the first steps in trying to understand its function. We have shown that combining a grammar-based specimen model with an imaging model is useful to automatically obtain quantitative information for biological structures in microscopic image stacks. The model is used to represent and quantify the fungal structure, augment computer software to automatically identify unknown samples, and also serves as a new educational tool for scientists and students. The L-system model developed provides

quantitative information about the biological structure. From this it will be possible to link these data with other complex data sets such as gene expression or metabolite profiles.

This version of the model encodes the statistical variation of geometric parameters for different species, however it does not use an elaborate structure for the spores. Currently, we use ellipses with various sizes, depending on the age of the spore – the older spores have longer length and larger radius. However, an important taxonomic characteristic is spore shape and future work will include modelling the spores according to their published descriptions (Simmons 2007).

Going beyond the form of a particular individual, we need to quantify the statistics for the range of form across individuals in a species, across groups of individuals and across species. Future studies will attempt to include environmental changes that affect the model parameters.

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