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Trends in Morphological Evolution in Homobasidiomycetes Inferred Using Maximum Likelihood: A Comparison of Binary and Multistate Approaches

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Abstract.— The homobasidiomycetes is a diverse group of macrofungi that includes mushrooms, puffballs, coral fungi, and other forms. This study used maximum likelihood methods to determine if there are general trends (evolutionary tendencies) in the evolution of fruiting body forms in homobasidiomycetes, and to estimate the ancestral forms of the homobasidiomycetes and euagarics clade. Character evolution was modeled using a published 481-species phylogeny under two character-coding regimes: additive binary coding, using DISCRETE, and multistate (five-state) coding, using MULTISTATE. Inferences regarding trends in character evolution made under binary coding were often in conflict with those made under multistate coding, suggesting that the additive binary coding approach cannot serve as a surrogate for multistate methods. MULTISTATE was used to develop a "minimal" model of fruiting body evolution, in which the 20 parameters that specify rates of transformations among character states were grouped into the fewest possible rate categories. The minimal model required only four rate categories, one of which is approaching zero, and suggests the following conclusions regarding trends in evolution of homobasidiomycete fruiting bodies: (1) there is an active trend favoring the evolution of pileate-stipitate forms (those with a cap and stalk); (2) the hypothesis that the evolution of gasteroid forms (those with internal spore production, such as puffballs) is irreversible cannot be rejected; and (3) crustlike resupinate forms are not a particularly labile morphology. The latter finding contradicts the conclusions of a previous study that used binary character coding. Ancestral state reconstructions under binary coding suggest that the ancestor of the homobasidiomycetes was resupinate and the ancestor of the euagarics clade was pileate-stipitate, but ancestral state reconstructions under multistate coding did not resolve the ancestral form of either node. The results of this study illustrate the potential sensitivity of comparative analyses to character state definitions. [Character coding; comparative methods; DISCRETE; fungi; morphology; MULTISTATE; phylogeny.]

The homobasidiomycetes is a diverse clade of fungi that contains roughly 16,000 described species (Kirk et al., 2001). Fruiting bodies of homobasidiomycetes include gilled mushrooms, puffballs, bracket fungi, coral fungi, and other forms (Fig. 1). Traditionally, the higher taxa of homobasidiomycetes were grouped according to the gross morphology of their fruiting bodies. Anatomical and molecular evidence indicates that there has been extensive convergence in fruiting body morphology, however (e.g., Jülich, 1981; Oberwinkler, 1985; Hibbett and Thorn, 2001; Kirk et al., 2001).

Many studies have focused on resolving the patterns of evolution of fruiting body forms in homobasidiomycetes (e.g., Bruns et al., 1989; Hibbett et al., 1997; Binder and Bresinsky, 2002; Humpert et al., 2001). The goal of the present study was to determine if there are "trends" (i.e., general evolutionary tendencies; McShea, 1994, 1996) in the evolution of fruiting body forms in homobasidiomycetes. For this purpose, fruiting body morphology was coded as a discrete character and maximum likelihood (ML) methods (Pagel, 1994, 1997, 1999; Schluter, 1995; Schluter et al., 1997) were used to test alternative models of fruiting body evolution. The parameters of such models specify the rates of change between pairs of character states. This study aimed to determine if there are significant differences among rate parameters, which would suggest the existence of directionality in fruiting body evolution.

The ML approach has become popular for comparative studies of morphological and ecological characters, including analyses of directionality, character correlations, and ancestral states (e.g., Lutzoni and Pagel, 1997; Cunningham, 1999; Mooers and Schluter, 1999; Ree and Donoghue, 1999; Hibbett et al., 2000; Hibbett and Donoghue, 2001; Lutzoni et al., 2001; Hibbett and Binder, 2002; Krüger and Davies, 2002; Oakley and Cunningham, 2002). A critical step in all such analyses is character coding. Character state definitions constitute central assumptions of comparative analyses, which may have large effects on inferences about evolutionary processes. Nevertheless, there has been little discussion of the methods or criteria for coding characters for comparative analyses, and few comparative studies have examined the sensitivity of their results to alternative coding regimes (for an exception, see Hibbett and Donoghue, 2001). The goals of character coding for phylogenetic reconstruction are not necessarily the same as those for comparative analyses. In phylogenetic reconstruction, the goal of character coding is to identify homologies. In comparative analyses, however, the goal of character coding is to recognize functionally equivalent structures (i.e., structures that experience similar selective forces), whether they are homologous or not. Harvey and Pagel (1991: p. 31) called this the "guild school" approach to character state definition.

Most comparative ML analyses of discrete characters have used Pagel's (1994) computer program DISCRETE, which allows only binary (two-state) character coding. Harvey and Pagel (1991: p. 78) suggested that this does not pose a problem for analyses of characters with more than two states, because "any multicategory discrete variable can always be represented as a set of dichotomous classifications, each one representing the presence or absence of a particular state" (for an example in fungi,



FIGURE 1. Fruiting body forms in homobasidiomycetes, and models of evolution inferred under binary codings A to E. Thickness of arrows indicates "directionality" of transformation bias in unrestricted model; thicker lines indicate larger rate parameters, but thickness of lines only indicates relative values within a pair of rate parameters, not absolute values. Solid black and dashed, dark gray arrows indicate significant transformation bias (P < 0.001, P < 0.05); light gray lines indicate nonsignificant transformation bias. A, Binary coding A (0 = pileate-stipitate). B, Binary coding B (0 = gasteroid). C, Binary coding C (0 = resupinate). D, Binary coding D (0 = pileate-sessile). E, Binary coding E (0 = coralloid-clavarioid). The ordering of fruiting body forms in the right-hand column (state 1) has no significance.

see Lumbsch et al., 2002). Such an approach is analogous to the use of additive binary coding in phylogenetic analysis. Recently, Pagel has developed a set of programs called MULTISTATE (distributed by M. Pagel; http://sapc34.rdg.ac.uk/meade/Mark/main.html) that enable comparative analyses of characters with up to six states. Like DISCRETE, MULTISTATE employs a Markov process and estimates a model of evolution, in which the parameters specify rates of transformations between character states.

This study used both MULTISTATE and DISCRETE to develop models of fruiting body evolution in homobasidiomycetes. As described below, fruiting body forms in homobasidiomycetes can be divided into five major morphotypes, which may represent functional "guilds." MULTISTATE is attractive for analyses of fruiting body evolution in homobasidiomycetes, because it makes it possible to develop models that treat each morphotype as a unique character state, rather than forcing dissimilar, putatively nonequivalent forms into the same character state. In practice, however, MULTISTATE has the drawback that it involves the estimation of a greater number of rate parameters than DISCRETE. For example, while a model of evolution for a binary character has only two rate parameters, a model for a five-state character has 20 rate parameters. Therefore, MULTISTATE requires larger phylogenetic trees to evaluate models than DISCRETE. In the documentation to MULTISTATE, Pagel suggests that for each parameter to be estimated there should be at least 10 species in the phylogenetic tree. According to these guidelines, a tree of at least 200 species would be needed to estimate the parameters of a five-state model.

The need for large phylogenetic trees is a potential barrier to the use of MULTISTATE, not only because large datasets pose analytical challenges, but also because some clades may not contain enough species to allow evaluation of parameter-rich models. Therefore, some researchers might prefer to employ additive binary character coding even when the character of interest occurs in more than two states. However, such an approach assumes that results obtained under additive binary coding would be congruent with those obtained under multistate coding. This study provides an empirical test of whether such an assumption is warranted.

Diversity and Evolution of Homobasidiomycete Fruiting Bodies

The classification of homobasidiomycetes is currently being revised, largely on the basis of molecular phylogenies. Hibbett and Thorn (2001) reviewed the literature on homobasidiomycete phylogeny and suggested that the group can be divided into at least eight independent clades, to which they gave informal names (e.g., euagarics clade, polyporoid clade, etc.). Major studies that have appeared since the review by Hibbett and Thorn include those of Binder and Hibbett (2002), Hibbett and Binder (2002), Langer (2002), Larsson (2002), and Moncalvo et al. (2002). For the most part, these studies have upheld the higher-level classification suggested by Hibbett and Thorn, although several additional clades have been detected and some analyses suggest that the polyporoid clade is not monophyletic (Hibbett and Binder, 2002; Langer, 2002; Larsson, 2002).

Fruiting bodies of homobasidiomycetes can be divided into five major morphotypes (Fig. 1): (1) Pileatestipitate forms are divided into a cap and a stalk, and may have one or more "veils" that enclose the developing hymenophore (the spore-bearing surfaces). The hymenophore of pileate-stipitate forms is often composed of gills, but it may also be poroid, toothed, ridged, or smooth. (2) Gasteroid forms produce spores internally and include puffballs, bird's nest fungi, earthstars, false truffles, and stinkhorns. (3) Resupinate forms lie flat on their substrates and range from thin, cobwebby fruiting bodies, to more robust fleshy or crustlike forms, with smooth, poroid, toothed, or ridged hymenophores. (4) Pileate-sessile forms include bracket fungi and other forms that have a cap but no stalk. (5) Coralloid-clavarioid forms are club or candelabra-shaped. There are intermediates between these categories, such as "effusedreflexed" forms, which have both a resupinate portion and a pileate portion, and some taxa have unique fruiting body types that are difficult to place in a category (e.g., the "cauliflower fungus" Sparassis). Nevertheless, the categories given above can be used to describe the vast majority of homobasidiomycete fruiting bodies. This study focused particularly on prior hypotheses regarding the evolution of pileate-stipitate forms, gasteroid forms, and resupinate forms. There were no prior hypotheses regarding the evolution of pileate-sessile or coralloid-clavarioid forms. An overview of the phylogenetic diversity of pileate-stipitate forms, gasteroid forms, and resupinate forms, and hypotheses concerning their evolution is provided below.

Pileate-stipitate forms are by far the most common fruiting body type in the homobasidiomycetes. Based on figures in the Dictionary of the Fungi, 9th edition (Kirk et al., 2001), there are roughly 12,000 described species (75%) of homobasidiomycetes that have pileate-stipitate fruiting bodies. Pileate-stipitate forms occur in each of the eight major clades of homobasidiomycetes recognized by Hibbett and Thorn, with the majority (about 8700 species) in the euagarics clade. The preponderance of pileate-stipitate forms among the homobasidiomycetes could suggest that there is a "driven" trend (McShea, 1994, 1996) toward this type of fruiting body morphology. To test whether such a trend exists, this study addressed whether the rate of transformations from nonpileate-stipitate forms to pileate-stipitate forms exceeds the rate of transformations in the reverse direction. If so, then this would suggest that natural selection tends to favor the evolution of pileate-stipitate fruiting bodies in homobasidiomycetes.

Gasteroid forms include only about 1200 described species (8%) of homobasidiomycetes (Hawksworth et al., 1995), which are distributed across five of the eight major clades of homobasidiomycetes recognized by Hibbett and Thorn (2001). The unifying feature of gasteroid forms is that the spores are produced internally and in most

cases they are not forcibly discharged from the cells that produce the spores. In contrast, all homobasidiomycetes that produce spores on the surfaces of their fruiting bodies have a complex forcible spore discharge mechanism called ballistospory (with rare exceptions; e.g., *Entomocorticium*, *Digitatispora*).

Structural features associated with ballistospory include asymmetric spores with a process (the hilar appendage) at the base of the spore, short, curved stalks on which the spores are produced, and production of a droplet of fluid at the base of the spore at the time of discharge. The vast majority of gasteroid forms all lack this combination of features, although a few gasteroid forms have retained ballistospory (e.g., Desjardin, 2003). It has been suggested that the loss of ballistospory is irreversible, on the grounds that such a complex feature might easily be lost but could not be regained (Thiers, 1984; Hibbett et al., 1997). Therefore, the evolution of gasteroid forms, including the loss of ballistospory, could provide an example of an evolutionary constraint. To test this hypothesis, this study evaluated models of evolution in which the rate of transformations from gasteroid forms to forms with forcible spore discharge approaches zero.

Resupinate forms include roughly 2000 to 2400 species (13% to 15%), which occur in every major clade of homobasidiomycetes recognized by Hibbett and Thorn (2001) and Larsson (2002), as well as the closely related "jelly fungi," Auriculariales, Dacrymycetales, and Tremellales (Parmasto, 1997; Larsson, 2002; Hibbett and Binder, 2002). Hibbett and Binder (2002) performed comparative analyses of the evolution of resupinate forms in homobasidiomycetes using a data set of 464 species of homobasidiomycetes and 17 jelly fungi, with fruiting body morphology coded as a binary character (0 =resupinate; 1 =all others). Hibbett and Binder performed their analyses with DISCRETE, using nine different phylogenetic trees that varied in topology, branch lengths, and sampling regimes, which are all known to influence estimates of rate parameters (Cunningham, 1999; Mooers and Schluter, 1999; Omland, 1999; Ree and Donoghue, 1999; Huelsenbeck et al., 2000; Lutzoni et al., 2001; Salisbury and Kim, 2001; Pagel and Lutzoni, 2002; Huelsenbeck et al., 2003). In all of their analyses, the rate of transformations from resupinate to nonresupinate forms was estimated to be at least three times greater than the rate of transformations in the reverse direction, and a null model of evolution with equal transformation rates was rejected. Hibbett and Binder therefore concluded that resupinate fruiting body forms are evolutionarily more labile than nonresupinate forms, and that there is a driven trend toward the evolution of nonresupinate fruiting body morphologies in homobasidiomycetes. Hibbett and Binder argued that resupinate forms are qualitatively simple, relative to nonresupinate forms, and therefore they suggested that there is a driven trend toward increasing morphological complexity in homobasidiomycetes. The results of Hibbett and Binder (2002) were robust to variation in phylogenetic trees, but the binary character coding that they employed is

a poor reflection of the diversity in fruiting body forms in homobasidiomycetes. This study tested the results of Hibbett and Binder (2002) using multistate coding.

METHODS

Trees and Character Coding

Analyses were performed using a tree from the study of Hibbett and Binder (2002) that was obtained with an equally weighted parsimony analysis, with branch lengths estimated using ML. The data set of Hibbett and Binder (2002) included overlapping nuclear and mitochondrial small subunit and large subunit rDNA sequences. One hundred seventeen species were represented by all four regions, 78 species were represented by three regions, and 12 species were represented by two regions. All species in the data set were represented by nuclear large subunit rDNA sequences (1033 bp), which were used to estimate branch lengths. The total aligned length was 3800 bp, including 2262 variable positions and 1605 parsimony-informative positions. Nodal support was estimated for the present study using bootstrapped equally weighted parsimony, with 500 replicates, each with one heuristic search with a random taxon addition sequence and TBR branch swapping, with MULTREES off.

By using only a single topology, these analyses effectively treat the phylogeny as known. In the study of Hibbett and Binder (2002), from which the tree used here was drawn, nine different trees were sampled, which varied in topology, taxon sampling regime, and branch length estimates. Selection of alternative trees had only a modest impact on conclusions regarding trends in character evolution. The results of Hibbett and Binder (2002) can be contrasted with those of Lutzoni et al. (2001), who used ML methods with binary coding to study the evolution of lichen symbioses in ascomycetes. Lutzoni et al. (2001) found that rates of transformations between lichenized and nonlichenized states were quite sensitive to variation in tree topology, and a general trend could only be detected using a large number of trees obtained with a Bayesian Markov chain phylogenetic analysis. In future analyses, it would be valuable to study evolution of homobasidiomycete fruiting bodies using multiple tree topologies, as was done by Lutzoni et al. (2001). In particular, it would be useful to examine whether estimates of transformation rates obtained under multistate coding are more or less robust to variation in tree topology than estimates obtained under binary coding. Such analyses are beyond the scope of the present study, which addresses the impact of variation in character coding when the topology is held constant.

Taxa were coded using either two or five discrete states (see Figs. 1–3). Five alternative binary character codings were employed, in which the "zero" state was either pileate-stipitate (binary coding A), gasteroid (binary coding B; none of the gasteroid forms in this study are ballistosporic), resupinate (binary coding C), pileate-sessile (binary coding D), or coralloid-clavarioid (binary coding E). In the five-state coding, the states were 0 = resupinate (148 species, 33%), 1 = pileate-sessile (88 species, 18%), 2 = pileate-stipitate (209 species, 43%), 3 = coralloid or clavarioid (14 species, 3%), and 4 = gasteroid (22 species, 5%). Results for binary coding 3 were taken from Hibbett and Binder (2002).

Binary Analyses

Binary analyses were performed using DISCRETE v. 4.0 running under Connectix Virtual PC emulation on a 477 MHz Macintosh G4 computer. To assess whether there are asymmetries in the rates of transformations between the states of binary characters, models of evolution were estimated with no restrictions on the parameter values (rates of gains and losses) and the likelihood was obtained. Next, the gain and loss rate parameters were restricted to being equal, and the model parameters and likelihood were estimated again. The competing models are considered to be "nested," because the restricted model (with both parameters defined using a single rate class) is a special case of the unrestricted model (with two rate classes). The test statistic used to determine if the unrestricted model provides a significantly better fit to the data is equal to twice the difference in log likelihoods and is χ^2 distributed with one degree of freedom (Pagel, 1997, 1999). In these and all other analyses, the program was run at least five times.

To test the hypothesis that the evolution of gasteroid forms is irreversible, the rate of transformations from gasteroid to nongasteroid forms ($0 \rightarrow 1$ in binary coding B) was restricted to zero, and the likelihood was obtained and compared to that obtained under the unrestricted model. In this case, the models are not nested, so a difference of two units in log likelihood scores was used as a rule-of-thumb criterion for "strong" support for one model over another, following Pagel (1999) and Mooers and Schluter (1999).

Multistate Analyses

Three types of analyses were performed with the fivestate character coding using MULTISTATE, again running under Virtual PC: (1) paired asymmetry tests; (2) tests of the irreversibility of gasteroid forms; and (3) minimal model analyses.

Paired asymmetry tests.—Analyses to detect asymmetries in the rates of transformations between all pairs of character states were performed using essentially the same approach as for binary characters. For a character with five states, there are 20 parameters that specify rates of transformations between states (q01, q10, q02, q20, etc.), and 10 possible pairwise tests of asymmetry. In each test, two parameters were restricted that specify rates of transformations between a pair of states to being equal (e.g., q03, q30) and the likelihood score was obtained and compared to the likelihood score obtained for the unrestricted model using the likelihood ratio test with 1 d.f.

Tests of irreversibility of gasteroid forms.—To test the hypothesis that the evolution of gasteroid forms is

irreversible, models were generated in which all four parameters that specify rates of transformation from gasteroid to nongasteroid forms (q40, q41, q42, q43) were restricted to 0.000001 (restricting these parameters to zero caused the program to terminate) and the likelihood was compared to that obtained with the unrestricted model. Again, a difference of two units in log-likelihood scores was used as a rule-ofthumb criterion for "strong" support of one model over another.

Minimal model analyses.—The goal of these analyses was to develop a "minimal" model of evolution of fruiting body forms, in which the 20 parameters that describe rates of transformations between the five character states are grouped into the fewest possible rate classes. To estimate the minimal model, nested models of evolution that have different numbers of rate classes were generated, and likelihood ratio tests were used to select the simplest model that could not be rejected. This approach is similar to the use of hierarchical likelihood ratio tests to choose among models of molecular evolution (Posada and Crandall, 1998).

In developing the minimal model, it was assumed that the evolution of gasteroid forms is irreversible. Thus, the search for the minimal model started with a model that has 17 rate classes, with the parameters q40, q41, q42, and q43 combined into a single rate class that was set to 0.000001. The remaining parameters were ordered according to their values in the 17-rate model, the two most similar parameters were pooled (i.e., restricted to take the same value), and a 16-rate model was estimated. The 16-rate model was compared to the 17-rate model using a likelihood ratio test with 1 d.f. Next, a 15-rate model was estimated by pooling the closest parameters in the 16-rate model, and so on. When a model was rejected, alternative groupings of parameters were examined (while holding the number of rate classes constant). The search continued until it was impossible to merge rate classes without rejecting the resulting model.

Ancestral State Reconstructions

The character states of two nodes, representing the ancestor of the homobasidiomycetes and the ancestor of the euagarics clade, were estimated using the "local" method as defined by Pagel (1999). In this approach, a node is fixed at a given state, and the model parameters and likelihood are estimated; next, the node is fixed at an alternative state and the model and likelihood are estimated again. A difference of two units in loglikelihood scores is taken as a rule-of-thumb criterion for "strong" support for one state over another (Mooers and Schluter, 1999; Pagel, 1999). Ancestral state reconstructions under binary coding were performed using DISCRETE, with unrestricted parameter values. Ancestral state reconstructions under multistate coding were performed using MULTISTATE. For the latter, parameter values were either unrestricted or pooled according to the minimal model.

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		contected mos	aci		Restricted	intotaci			
Character coding	$\alpha \; (0 \to 1)$	$\beta \; (1 \to 0)$	-logL	Restriction	$\alpha \; (0 \to 1)$	$\beta \; (1 \to 0)$	-logL	$2\Delta logL$	Rejected?
Binary coding A $(0 = pileate-stipitate)$	3.655	2.267	439.002	$\alpha = \beta$	2.620	2.620	441.004	4.004	Yes ^a
Binary coding B $(0 = \text{gasteroid})$	1.680	0.413	135.099	$\alpha = \beta$	0.389	0.389	135.568	0.938	No
				$\alpha = 0$	0.000	0.341	136.350	2.502	No
Binary coding C ($0 = \text{resupinate}$)	4.385	1.379	405.013	$\alpha = \beta$	2.392	2.392	414.590	19.154	Yes ^b
Binary coding D ($0 = pileate-sessile$)	11.695	2.702	423.646	$\alpha = \beta$	2.450	2.450	436.614	25.936	Yes ^b
Binary coding $E(0 = \text{coralloid-clavarioid})$	10.795	0.460	108.879	$\alpha = \beta$	0.349	0.349	115.227	12.696	Yes ^b

TABLE 1. Tests of asymmetry in transformation rates under binary character coding.

 $^{a}P < 0.05; ^{b}P < 0.001.$

RESULTS

The Phylogeny

The phylogenetic tree used to model character evolution and the distribution of character states among terminal taxa are shown in Figure 2. Eleven major clades of homobasidiomycetes are resolved (Fig. 2). Bootstrap support values for these groups are generally low, but their monophyly has been strongly supported in earlier analyses (except for the polyporoid clade) that use multiple genes or that have more limited taxon sampling (Hibbett and Thorn, 2001; Binder and Hibbett, 2002; Larsson, 2002). The numbers of species in each clade are roughly comparable to their estimated proportions (Hibbett and Thorn, 2001; Hibbett and Binder, 2002).

Binary Analyses

Analyses under four of the five binary codings suggest that there is an asymmetry in the rate of transformations between character states (Fig. 1, Table 1). Under binary coding A (0 = pileate-stipitate), the unrestricted model of evolution suggests that the rate of transformations from pileate-stipitate forms to non-pileate-stipitate forms is about 1.6 times the rate of transformations in the reverse direction. Under binary coding B (0 =gasteroid), the rate of transformations from gasteroid to nongasteroid forms is about four times greater than the rate of transformations in the reverse direction in the unrestricted models, but the null model of equal rates could not be rejected. In addition, the –log-likelihood score obtained when the rate of transformations from gasteroid to nongasteroid forms was set to zero is only marginally worse than that obtained under the unrestricted model ($\Delta \log L < 2.0$), suggesting that the hypothesis that evolution of the gasteroid condition is irreversible cannot be rejected. Under binary codings C to E, respectively, there appear to be trends toward the evolution of nonresupinate forms, non-pileate-sessile forms, and non-clavarioid-coralloid forms; in each of these cases, the model with equal rates of transformations was rejected.

Multistate Analyses

Unrestricted model and paired asymmetry tests.—Unrestricted parameter values in the multistate model ranged over several orders of magnitude, from 6.87056 (pileatesessile \rightarrow pileate-stipitate; q12) to 0.000033 (gasteroid \rightarrow pileate-stipitate; q42). Nevertheless, only one asymmetry test returned a significant result: The unrestricted model suggests that the rate of transformations from pileate-sessile to pileate-stipitate (q12) forms is about 2.5 times greater than the rate of transformations in the reverse direction (q21), and a null model of equal rates was rejected (P < 0.05; Fig. 3, Table 2). There is also weak support (0.10 < P < 0.25) for asymmetries involving transformations between coralloid-clavarioid forms and either gasteroid (q34, q43), resupinate (q30, q03), or pileate-stipitate (q32, q23) forms. In each of these pairs of character states, the rate of transformations away from coralloid-clavarioid forms appears to be greater than the rate of transformation toward coralloid-clavarioid forms (Table 2; Fig. 3).

Tests of irreversibility of gasteroid forms.—The unrestricted model of evolution suggests that the rate of transformations from gasteroid forms to all nongasteroid forms (q40, q41, q42, q43) is greater than zero (Table 2). Nevertheless, the restricted model that suggests that evolution of gasteroid forms is irreversible (q4i = 0) could not be rejected ($\Delta \log L < 2$; Table 2).

Minimal model analyses.—Twenty-one individual models were evaluated in the course of developing the minimal model (Table 3). None of the models with seven or more rate classes could be rejected, but some models with four to six rate classes were rejected. One three-rate model was estimated and rejected, but two other threerate models could not be estimated (the program was terminated after running overnight). The search resulted in two models with four rate classes that could not be rejected. One of the four-rate models is marginally superior to the other according to likelihood and is therefore designated the minimal model (Table 3). In this model, the fastest rate class includes three parameters, which specify the rate of transformations from pileate-sessile forms to pileate-stipitate forms (q12), pileate-sessile forms to resupinate forms (q10), and coralloid-clavarioid forms to resupinate forms (q30). The second rate class includes 5 parameters; the third rate class includes 2 parameters; and the fourth rate class includes 10 parameters, including those specifying rates of transformations from gasteroid to nongasteroid forms (q40, q41, q42, q43; Table 3, Fig. 3). Thus, the minimal model suggests that 10 kinds of transformations occur at rates approaching zero. In the suboptimal four-rate model, the parameter specifying the rate of transformations from coralloid-clavariod



FIGURE 2. Phylogenetic relationships of homobasidiomycetes and distribution of fruiting body forms. Symbols next to terminal taxa indicate fruiting body forms, and symbols on branches indicate levels of bootstrap support (see figure for key to symbols). Arrowheads indicate nodes that were used in ancestral state estimates. Node 1 is the ancestor of the homobasidiomycetes and node 2 is the ancestor of the euagarics clade. The tree was inferred using a data set of 3800 bp of nuclear and mitochondrial rDNA sequences, which was analyzed with a two-step heuristic analysis using equally weighted parsimony. In the first step, 1000 heuristic searches were performed, with random taxon addition sequences and TBR branch swapping, keeping two trees per replicate. In the second step, the shortest trees were used as starting trees for TBR branch swapping with MAXTREES set to 10,000. This is 1 of 10,000 equally parsimonious trees (23,536 steps, CI = 0.174). Nodal support was estimated with bootstrapped equally weighted parsimony, using 500 replicates, each with one random taxon addition sequence, TBR branch swapping, and MULTREES off. (*Continued*)



FIGURE 2. (Continued).



FIGURE 3. Models of evolution inferred under multistate coding. a, Summary of results of paired asymmetry tests. Thickness of arrows indicates directionality of transformation bias between pairs of character states in unrestricted model; thicker lines indicate larger rate parameters; thickness of lines only indicates relative values within a pair of rate parameters, not absolute values of rate parameters. Solid black arrows indicate significant transformation bias (P < 0.05), dashed, dark gray arrows indicate weakly supported trends (0.10 < P < 0.25) in transformation bias; solid light gray arrows indicate non-significant transformation bias. b, Minimal model of evolution of fruiting body forms. Solid black arrows indicate rate class 1 (fastest); dashed, dark gray arrows indicate rate class 2; solid light gray arrows indicate rate class 3; no arrow indicates rate class 4 (approaching 0).

	$\begin{array}{l} q40 = q41 = q42 = \\ q43 = 0.000001 \end{array}$	3.407927	0.601039	0.285534	0.000030	5.031758	6.513865	1.188604	0.000012	0.133997	2.811194	0.068607	0.600561	4.212099	0.000657	2.211484	3.441386	0.00001	0.00001	0.00001	0.00001	428.688071	2.392208	No ^c
	q34 = q43	3.581830	0.607618	0.251663	0.000524	4.724671	6.827966	1.390598	0.000156	0.134231	2.716885	0.005521	0.827602	5.429840	0.002407	1.922075	2.357781	0.000077	0.000858	0.000098	2.357781	428.556274	2.128614	Yes^{a}
	q24 = q42	3.439191	0.623956	0.306226	0.000013	4.955234	6.516469	1.230157	0.000532	0.128539	2.735479	0.067396	0.725970	3.499032	0.001602	1.952628	4.179818	0.412950	0.001231	0.725970	0.000141	427.574500	0.165066	No
	q23 = q32	3.313270	0.728729	0.327846	0.000059	4.950242	6.463212	0.831594	0.000215	0.186212	2.734989	0.263576	0.718373	3.881279	0.000036	0.263576	4.781633	0.000765	0.000960	1.636100	0.000572	428.287110	1.590286	$\mathrm{Yes}^{\mathrm{a}}$
	q14 = q41	3.474402	0.613981	0.307295	0.000014	4.857717	6.710448	1.244595	0.000331	0.116235	2.706276	0.049707	0.739628	3.426927	0.000628	2.055127	4.125695	0.994906	0.000331	0.000803	0.000763	427.475701	0.032532	No
er restrictions	q13 = q31	3.293991	0.693745	0.407584	0.000138	4.965751	6.521071	0.811261	0.000074	0.130928	2.700219	0.170539	0.730568	2.883804	0.811261	1.968105	4.235368	0.971290	0.002052	0.000235	0.000342	427.865771	0.747608	No
Paramet	q12 = q21	2.712097	1.415371	0.448508	0.00001	5.498009	3.359966	0.735692	0.000034	0.001592	3.359966	0.201483	0.700001	3.150839	0.001588	2.244471	4.330167	0.004241	0.005394	1.082110	0.001160	430.294671	5.605408	Yes^b
	q04 = q40	3.450261	0.600362	0.304446	0.000042	5.035565	6.611164	1.203977	0.000310	0.139583	2.780084	0.075614	0.721149	3.627497	0.002637	1.900437	4.100154	0.000042	0.007722	1.084270	0.003202	427.626646	0.269358	No
	q03 = q30	3.212474	0.743081	0.474503	0.000190	5.046136	6.381723	0.645691	0.000021	0.133097	2.698514	0.172391	0.739575	0.474503	0.000444	2.209341	5.213918	1.347415	0.000486	0.004613	0.001520	428.515136	2.046338	$\mathrm{Yes}^{\mathrm{a}}$
	$q^{02} = q^{20}$	3.913107	0.217168	0.250589	0.000357	4.666707	7.789283	1.406053	0.000371	0.217168	2.474743	0.000327	0.758142	3.534520	0.000895	2.020492	4.071211	1.006691	0.004612	0.000225	0.001562	427.676154	0.368374	No
	q01 = q10	3.930697	0.364511	0.223053	0.000086	3.930697	7.564464	1.498293	0.002675	0.215438	2.411768	0.001149	0.768645	3.983402	0.000271	1.875320	3.985990	1.074571	0.001296	0.001211	0.002032	427.791122	0.59831	No
	None	3.560685	0.554681	0.279362	0.000411	4.852035	6.870562	1.373391	0.001958	0.108550	2.702828	0.005330	0.746162	3.476394	0.009280	2.039304	4.058055	1.002416	0.001315	0.000033	0.000306	427.491967		
	Parameter	q01	q02	q03	q04	q10	q12	q13	q14	q20	q21	ą23	q24	q ₃₀	q31	q32	q34	q40	q41	q42	q43	$-\log L$	$2\Delta logL$	Rejected?

TABLE 2. Paired asymmetry tests (columns 2 to 12) and test of irreversibility of gasteroid forms (column 13) under five-state coding.

 ${}^{a}P = 0.10 - 0.25$; ${}^{b}P = 0.01 - 0.025$; ${}^{c}\Delta \log L > 2$.

E 3. Steps in the development of the minimal model under five-state coding (assuming that $q4i = 0.00001$).
TABL

	3a 3a	<u>.</u>		I				I		-					I										
	8	о ,		4.466563		1		1.621090		1						0.00001							43.955991 -	25.68866 -	;
	4	4	6 867030	10/2000		3 336486	00100000				0.578330	0000 1000				0.00001							33.041993 44	4.244906	
	4	+		5.800533			3.077353				0.620040	0E//70:0				0.00001							31.624262 4.	1.409444	ŀ
	4	4		5.611262	<u> </u>		101100 0	#61100°C			0.644738	00 755000				0.00001							31.111661 4	0.384242	
	c,	0	6 310503	010010	610071.0		0.07074	4/6/40°C			0.615223	7776100				0.00001							430.919540 4	2.94327	
	5	c	6 187060	6.100.000	cccn/T.c		3.225267			0.886790						0.00001							432.062751	5.229692	
	6	٥	6 800176	E 242202	707647.0		3.413557		1 866648	OLONOO-T	0 508001	1000/010				1000000							429.447905	0.435882	
S	6	0	alues 8.478858	200000000	/#1070.0	5 340839	10001010	3.752309	4 168408	COTOOT T					0.00001								447.062743	35.665558	
rate classe	7	\ \ 	d parameter v	5.074100	C014/C.C	3 749610	010/1 00	3.032780	1 880191	1/10001	0 575735	0070 10:0				0.00001							429.229964	0.021898	
No.	8	0	Estimate	5.512662	4.948370	3 776376	0.000 1.00	3.034626	1 862036	000700-1	0 573465	0000 0000				0.00001							429.219015	0.000844	
	6	6	6 613073	5.506296	4.979967	3 774846		3.034616	1.803858	1.864647	0 573652	7000 /0:0				0.00001							2 429.218593	0.852342	
	10	TO	6 56/1/1	5.401150	4.201257	3 474822		2.996587	2.261457	1.344459	0.596820	07007010	0.269814				0.00001						3 428.792422	0.188198	
	11		6 408365	5.153623	4.209058	3 404827	Terror of the	2.845208	2.244686	1.107917	0.607181	10170000	0.304219	0 104618	0101010			0.00001					5 428.69832	0.022616	
	12	7	6 518806	5.063596	4.226004	3 415639	2001	2.815645	2.225505	1.190363	201003.0	0010000	0.291534	0.131367	0.067225			0.00001					4 428.68701	0.000162	
	13	CI	6 517710	5.061818	4.214931	3.439603	3.410915	2.815047	2.228350	1.187209	0.60003	~~~~~	0.292141	0.131609	0.067801			0.00001					8 428.68693	0.000052	
	14	14	6 576/38	5.063081	4.216801	3.438488	3.415451	2.815206	2.228972	1.190597	0.595744	0.600687	0.291245	0.131481	0.066803			0.000001					9 428.68690	0.000062	
	15	CI	6 571668	5.060173	4.208986	3.439779	3.410624	2.814865	2.230226	1.182049	0.597856	0.600700	0.293295	0.131563	0.068785	0.000007		100000	Toopoon				1428.686935	0.000322	
	16	TO	6 517684	5.062136	4.209021	3.440326	3.410278	2.814241	2.227794	1.182132	0.599697	0.600323	0.292509	0.132103	0.068787	0.000384	0.00003		0.00001				428.687100	0.001942	
	17	1/	6 513865	5.031758	4.212099	3.441386	3.407927	2.811194	2.211484	1.188604	0.601039	0.600561	0.285534	0.133997	0.068607	0.000657	0.000030	0.000012		0.00001			428.688071	1	
Data	nate parameters	parameters	112	q10	q30	q34	q01	q21	q32	q13	q02	q24	q03	q20	q23	q31	q04	q14	d40	q41 J	q42	q43	-logL	2ΔlogL	

^aParameters for these models could not be estimated.

	Ancestral states and -logL													
	No	de 1: Ancesto	r of homob	asidiomyce	tes	Node 2: Ancestor of euagarics clade								
Character coding and model	0	1	2	3	4	0	1	2	3	4				
Binary coding A $(0 = pileate-stipitate; 1 = others)$	445.127	439.004ª	_	_	_	439.008ª	444.475	_	_	_				
Binary coding B $(0 = \text{gasteroid}; 1 = \text{others})$	137.355	135.109ª	—	—	—	156.751	135.099ª	—	—	—				
Binary coding C ($0 = \text{resupinate};$ 1 = others)	405.057ª	408.094	—	—	—	418.744	405.014 ^a	—	—	—				
Binary coding D (0 = pileate-sessile; 1 = others)	426.101	423.714 ^a	—	—	—	427.277	423.750ª	—	—	—				
Binary coding E (0 = coralloid-clavarioid; 1 = others)	110.107	109.216 ^b	—	_	_	122.874	108.879ª	_	_	_				
Multistate coding ^c / unconstrained (20-rate) model	19.048	17.754	17.751	17.729	17.712 ^b	286.926	285.336	285.253	285.226	285.214 ^b				
Multistate coding ^c / minimal (4-rate) model	23.054	21.252	21.160	21.164	21.142 ^b	295.609	294.130	294.004	294.001	293.978 ^b				

TABLE 4. Support for alternative ancestral states under binary and multistate character coding.

^aBest; $\Delta logL > 2$.

^bBest; $\Delta logL < 2$, n.s.

^c0 = resupinate; 1 = pileate-sessile; 2 = pileate-stipitate; 3 = coralloid-clavarioid; 4 = gasteroid.

forms to resupinate forms (q30) is in the second-fastest rate class instead of the fastest rate class (Table 3). The two four-rate models are otherwise identical, and the estimated transformation rates are very similar (Table 3).

Ancestral State Reconstructions

Ancestral state analyses under four of the binary coding regimes provided strong support for the ancestral state of the homobasidiomycetes (Table 4). Analyses under binary coding C (0 = resupinate) suggest that the ancestor of the homobasidiomycetes was resupinate, whereas analyses under binary codings A, B, and D suggest that the ancestor was not pileate-stipitate, pileatesessile, or gasteroid (respectively), and analyses under binary coding E (0 = coralloid-clavarioid) were inconclusive. In sum, these results are consistent with the hypothesis that the ancestor of the homobasidiomycetes was resupinate. Under multistate coding with either the unconstrained or minimal model, the ancestral state with the best (lowest) log-likelihood score is gasteroid. However, the difference in log-likelihoods between the two states under both models is less than two units of loglikelihood (Table 4). Results of ancestral state analyses for the ancestor of the euagarics clade are similar to those for the ancestor of the homobasidiomycetes. Ancestral state analyses under all five binary codings are consistent with the view that the ancestor of the euagarics clade was pileate-stipitate, but both analyses under multistate coding are inconclusive (Table 4).

DISCUSSION

The goals of this study were to determine if there are trends in the evolution of fruiting body forms in homobasidiomycetes, and to compare results obtained using binary and multistate coding. As noted previously, the multistate approach should have greater utility for this purpose, because it allows the creation of models in which functionally equivalent forms are recognized as individual character states, whereas the binary approach forces nonequivalent forms to be lumped into the same character state. In other words, multistate methods should result in more realistic evolutionary models than binary methods, because they are based on more biologically meaningful character state definitions. However, the multistate approach has less statistical power than the binary approach, because it requires the estimation of a greater number of parameters. This weakness was evident in the analyses of ancestral states-the multistate analyses were inconclusive, whereas binary analyses provided strong support for the ancestral form of the homobasidiomycetes (resupinate) and the euagarics clade (pileate-stipitate; Table 4). Similarly, under multistate coding only one of the paired asymmetry tests returned a significant result (Fig. 3, Table 2), whereas four out of five analyses under binary coding returned a significant result (Fig. 1, Table 1).

Because of their greater statistical power, some researchers may prefer to employ additive binary coding for analyses of multistate characters rather than true multistate coding. However, the findings of this study indicate that conclusions about evolutionary trends obtained with binary character coding may often conflict with those obtained with multistate character coding (see below), so it should not be assumed that a series of analyses using additive binary coding can be a surrogate for a multistate analysis, contrary to the suggestion of Harvey and Pagel (1991).

Of the multistate models that were produced, the minimal model is preferred because it is the model with the fewest free parameters that adequately explains the observations (the phylogeny and the distribution of character states). A benefit of the minimal model analysis is that by grouping parameters into a small number of rate classes, it was possible to detect asymmetries in transformation rates that were not strongly supported in the paired asymmetry tests. One practical limitation of the minimal-model approach, however, is that many models need to be evaluated to find the optimal model, which is rather tedious at present. The strategy that was used here required the evaluation of 21 different models to identify the minimal model (Table 3). More exhaustive search strategies might lead to other models with four or fewer rate classes that explain the data as well as the model that was obtained here. Clearly, there is a need for new methods and computer algorithms that will make it possible to efficiently develop minimally parameterized models of evolution for multistate characters, as is already possible for molecular characters (Posada and Crandall, 1998).

The minimal model suggests that transformations among fruiting body forms in homobasidiomycetes can be grouped into four rate categories, one of which is approaching zero and may be considered to represent virtually impossible (or unique?) types of transformations. Asymmetries in transformation rates are evident for every pair of character states in the minimal model (Fig. 3). In addition, no two character states are linked to the other three states by the same set of transformation rate classes (Fig. 3). This pattern suggests that each fruiting body form (character state) has unique evolutionary tendencies, which are reflected by a unique set of probabilities of transformations to other forms. The evolutionary properties of each of the five fruiting body morphotypes are discussed in the following sections.

Pileate-sessile and coralloid-clavarioid forms.-Pileatesessile forms and coralloid-clavarioid forms appear to be the most labile fruiting body morphologies in homobasidiomycetes. These conclusions were supported by both the binary and multistate analyses (Figs. 1, 3). The congruence of the binary and multistate analyses with regard to these character states seems to be related to the relative frequency of the states under the different coding regimes. Coralloid-clavarioid forms are the least common state (3%) under both binary coding and multistate coding, and both binary and multistate analyses suggest that rates of transformations from coralloidclavarioid forms to all other forms (except pileate-sessile forms) are greater than rates of transformations in the reverse direction (Figs. 1, 3, Tables 1 to 3). Similar results were obtained for pileate-sessile forms, which include 18% of the taxa and are the second least common state under multistate coding

Pileate-stipitate forms.—Multistate and binary analyses suggested different conclusions regarding the evolution of pileate-stipitate forms. The model of evolution inferred under binary coding suggests that there is an active trend in transformations away from pileate-stipitate forms, but the multistate model suggests that rates of transformations from all forms (except gasteroid forms) toward pileate-stipitate forms are greater than the rates of transformations in the reverse direction (Figs. 1, 3, Tables 1 to 3). Thus, the multistate approach suggests that the pileate-stipitate form is a relatively stable fruiting body morphology, whereas the binary approach suggests that it is relatively labile. Again, the results appear to be a consequence of the relative frequency of the character state under the different coding regimes; pileate-stipitate forms are the minority state in binary coding (43%), but they are the most common state in multistate coding. Based on current estimates of diversity in homobasidiomycetes, the actual frequency of pileate-stipitate forms may be significantly greater than 43%, however (Kirk et al., 2001). As more representative data sets are developed, the support for a driven trend toward pileate-stipitate forms should become stronger.

Estimates of transformation rates provide insight into the relative stability of different forms, but they do not reveal the specific patterns of stasis and change in lineages over evolutionary time, which can only be inferred through ancestral state reconstructions or examination of the fossil record. In this study, the ancestral state of the euagarics clade was estimated to be pileate-stipitate using binary character coding, although the ancestral state could not be estimated using multistate coding (Table 4). The apparent stability of pileate-stipitate forms is also consistent with the fossil record of homobasidiomycetes. Although the fossil record of this group is very limited, it is noteworthy that all of the known fossils of complete homobasidiomycete fruiting bodies are pileate-stipitate forms that are thought to belong to the euagarics clade. These include a Cretaceous taxon, Archaeomarasmius leggeti, from Atlantic Coastal Plain amber (ca. 90 to 94 Mya; Hibbett et al., 1995), and several Miocene taxa from Dominican amber (ca. 15 to 20 Mya; Poinar and Singer, 1990; Hibbett et al. 1995; Hibbett et al., 2003). The euagarics clade includes the majority of pileate-stipitate homobasidiomycetes, and is here represented by 214 species, of which 160 species (75%) are pileate-stipitate (Fig. 2). Taken together, the results of ancestral state reconstruction under binary coding and the fossil evidence suggest that the pileatestipitate fruiting body form is the plesiomorphic condition of the euagarics clade and has been conserved in many lineages (Fig. 2).

Transformations from pileate-stipitate forms to coralloid-clavarioid or resupinate forms were in the rate category approaching zero. This is somewhat surprising, because there are both coralloid-clavarioid and resupinate forms nested within the euagarics clade (Fig. 2; Hibbett and Thorn, 2001; Moncalvo et al., 2002). The minimal model suggests that transformations from pileate-stipitate forms to coralloid-clavarioid or resupinate forms probably occurred via pileate-sessile intermediates (Fig. 2).

Gasteroid forms.—The evolution of gasteroid forms has been suggested to be irreversible because it involves loss of the complex mechanism of ballistospory (Thiers, 1984; Hibbett et al., 1997). Both binary and multistate analyses were unable to reject this hypothesis, which became an assumption of the minimal model (Table 3, Fig. 3). The minimal model suggests that transformations to gasteroid forms occur only via pileate-stipitate or coralloid-clavarioid precursors. Derivation of gasteroid forms from pileate-stipitate ancestors has been suggested previously, because many pileate-stipitate mushrooms pass through a developmental stage in which the hymenophore is enclosed in a cavity within the fruiting body—it is easy to envision how a gasteroid form could be derived from such an ancestor by paedomorphosis (Thiers, 1984; Bruns et al., 1989). It is less obvious how the ontogeny of a coralloid-clavarioid form could be modified to give rise to a gasteroid form. Nevertheless, ontogenetic studies of gasteromycete-like forms of the homobasidiomycete Lentinus tigrinus suggest that gasteroid forms could be derived from precursors that do not have an enclosed hymenophore at any stage of development (Hibbett et al., 1994). Within the homobasidiomycetes, transformations from coralloid-clavarioid forms to gasteroid forms are most likely to have occurred within the gomphoid-phalloid clade, which includes many coralloid forms as well as diverse gasteromycetes, such as earthstars, false truffles, and stinkhorns (Fig. 2; Hibbett and Thorn, 2001; Humpert et al., 2001).

Resupinate forms.—Results for resupinate forms were comparable to those for pileate-stipitate forms. Resupinate forms are the minority state in binary coding (33%), but are the second most common state in multistate coding. Analyses under binary coding suggest that there is an active trend away from resupinate forms, but analyses under multistate coding suggest that this bias is only evident in transformations from resupinate to pileate-stipitate forms (and these were placed in the second slowest rate category; Figs. 1, 3). For pileate-sessile or coralloid-clavarioid forms, rates of transformations toward resupinate forms are greater than rates of transformations in the reverse direction (Figs. 1, 3). Based on the multistate analysis, resupinate forms do not appear to be particularly labile, and there is no evidence of a driven trend toward the evolution of complex fruiting body forms in the homobasidiomycetes, which contradicts the conclusions of Hibbett and Binder (2002). These results demonstrate the potential pitfalls of relying on additive binary coding to model the evolution of multistate characters.

CONCLUSIONS

The existence of evolutionary trends, and the methods for their detection, have been the subject of extensive debate (e.g., McShea, 1994, 1996; Gould, 1997). The ML approach pioneered by Pagel (1994, 1997, 1999) provides estimates of rates of character state transformations, which can be used to identify evolutionary trends, as well as estimate ancestral states. The results of the present study suggest that such analyses may be strongly affected by variations in character coding. In defining character states for comparative analyses, researchers make strong assumptions about both the biology of the organisms under study, and the nature of the evolutionary process. Specifically, all organisms scored as having the same character state are assumed to be functionally equivalent with regard to the aspect of their biology that is being modeled (Harvey and Pagel, 1991), and evolution is assumed to amount to a Markov process of transformations among these states. Comparative analyses themselves do not test these assumptions, but that does not mean that they must be accepted uncritically. Indeed, a general implication of the present work is that comparative studies of discrete characters should incorporate sensitivity analyses that examine the impact of alternative character coding regimes. When results obtained under different coding regimes conflict, as in the present study, the character-state definitions themselves should be evaluated. Functional analyses of homobasidiomycete fruiting bodies might be able to refine the character-coding scheme used here, but this is an area of research that remains largely unexplored. Assuming that gross morphology reflects functional groups, then the multistate coding probably provides a more realistic model of fruiting body evolution than the binary codings. The multistate minimal model suggests the following conclusions regarding the dynamics of fruiting body evolution in homobasidiomycetes: (1) there is an active trend toward the evolution of pileate-stipitate forms; (2) the hypothesis of irreversibility of evolution of gasteroid forms cannot be rejected; (3) resupinate forms are not particularly labile; and (4) pileate-sessile and coralloidclavarioid forms are the most labile fruiting body forms in homobasidiomycetes. The first and third conclusions contradict results that are strongly supported under binary character coding.

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