

Mating Systems in True Morels (*Morchella*)

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SUMMARY	1
INTRODUCTION	1
SEXUAL REPRODUCTION IN ASCOMYCETES	2
MATING-TYPE STRUCTURES IN <i>MORCHELLA</i>	5
EVOLUTION OF MATING-TYPE GENES IN <i>MORCHELLA</i>	9
MATING SYSTEMS IN <i>MORCHELLA</i>	10
DOMINANT HETEROTHALLISM WITH A PREVALENT HAPLOID LIFESTYLE	11
SPATIAL COMPETITION AND SKEWED DISTRIBUTION OF MATING TYPES	12
IMPACT OF MATING TYPE ON THE ASEQUAL STAGE AND CONIDIAL PRODUCTION IN <i>MORCHELLA</i>	13
HYPOTHETICAL LIFE CYCLE OF <i>MORCHELLA</i>	14
MORELS AS CANDIDATE MODEL ORGANISMS IN STUDYING SEXUAL DEVELOPMENT IN ASCOMYCETES	17
CONCLUDING REMARKS	18
APPENDIX	18
ACKNOWLEDGMENTS	18
REFERENCES	19
AUTHOR BIOS	23

SUMMARY True morels (*Morchella* spp., Morchellaceae, Ascomycota) are widely regarded as a highly prized delicacy and are of great economic and scientific value. Recently, the rapid development of cultivation technology and expansion of areas for artificial morel cultivation have propelled morel research into a hot topic. Many studies have been conducted in various aspects of morel biology, but despite this, cultivation sites still frequently report failure to fruit or only low production of fruiting bodies. Key problems include the gap between cultivation practices and basic knowledge of morel biology. In this review, in an effort to highlight the mating systems, evolution, and life cycle of morels, we summarize the current state of knowledge of morel sexual reproduction, the structure and evolution of mating-type genes, the sexual process itself, and the influence of mating-type genes on the asexual stages and conidium production. Understanding of these processes is critical for improving technology for the cultivation of morels and for scaling up their commercial production. Morel species may well be good candidates as model species for improving sexual development research in ascomycetes in the future.

KEYWORDS mating type, heterothallism, unisexual reproduction, pseudohomothallism, spatial competition, skewed distribution, mitospore, asexual reproduction, genome analysis, evolution

INTRODUCTION

True morels (*Morchella* spp., Morchellaceae, Pezizomycotina, Ascomycota) are highly prized edible fungi, renowned for their aromatic and gustatory qualities. They are distinguished by a unique honeycomb-like hymenium of the hollow fruiting body and usually fruit in the spring (at least in the temperate zone), except for some autumnal

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species (1–3). Phenotypic plasticity and subtle morphological characters make morel species difficult to distinguish from each other (4, 5). Consequently, genealogical concordance phylogenetic species recognition (GCPSR) of multiple gene sequences (internal transcribed spacer [ITS], *RPB1*, *RPB2*, and *EF1- α*) is extensively used in *Morchella* identification (5–14). Three evolutionary clades are easily recognized: (i) the basal Rufobrunnea clade (blushing morels), (ii) the Esculenta clade (yellow morels), and (iii) the Elata clade (black morels), which also includes semifree capped morels (10). To date, 78 phylogenetically distinct species have been recognized worldwide in the genus *Morchella* (Fig. 1).

The key importance of morels lies not only in their nutritional and medicinal properties (15, 16) but also in their economical and scientific value (1, 17). Morel cultivation has been a research focus worldwide for more than 100 years, starting with an original report from 1882 on their outdoor cultivation in France (18). Artificial cultivation of *Morchella rufobrunnea* in the United States (19), and especially of *M. importuna*, *M. sextelata*, and *M. eximia* in China (17), has spurred morel research worldwide. Recently, important new insights have been gained into various aspects of morel biology, including their reproductive modes (20–25), interspecific hybridization (26–28), biological and physiological characteristics (29–32), cultivation conditions (33), trophic modes (34, 35), transcriptome analysis (36), nuclear and mitochondrial genome analysis (25, 37–40), and the microbial community dynamics associated with the cultivation of morels (41, 42). The accelerated research into morels makes it highly probable that additional morel species will be successfully cultivated in the future. In 2019, the morel cultivation area in China had already reached 8,000 ha. However, typically, half of the morels in cultivation fail to fruit or exhibit a low rate of reproduction because of the low quality of strains (e.g., strain degeneration), environmental factors, management measures, and other factors. Undoubtedly, even considering the available knowledge amassed from basic and applied studies, the biology and cultivation technology for morels are both far from being fully elucidated.

In this review, we summarize the current understanding of the structure and diversification of mating-type genes, sexual reproduction, sexual process, segregation and imbalance of mating-type genes, and influence of the mating-type (*MAT*) locus on the asexual stages and the production of conidia in *Morchella*. We also present a hypothetical life cycle for morels, highlighting outstanding questions in the field. Finally, we propose morels as candidate model species to elucidate sexual development in ascomycetes.

SEXUAL REPRODUCTION IN ASCOMYCETES

More than 100,000 species of fungi have been described to date (43), exhibiting various life cycles and strong reproductive plasticity. The mechanisms of sexual and asexual reproduction in fungi are very interesting and deeply studied (44). Unlike animals with sex chromosomes, sexual reproduction in ascomycetes is under the control of a single locus, the mating type (*MAT*) locus (45, 46), first described over a century ago by Blakeslee (47) in Mucorineae. In fungi, the chromosomes bearing mating-type genes are usually called mating-type chromosomes or sex-related chromosomes (48, 49). The mating type locus normally has two mating types present, which by convention are indicated *MAT1-1* and *MAT1-2* (50, 51), also with alternative terminologies used in some species, such as *mat A* and *mat a* (e.g., in *Neurospora*) or *mat*[–] and *mat*⁺ (e.g., in *Podospora*) (52). These genes, located on the *MAT* locus, share little homology and are not true alleles but rather are termed “idiomorphs” (53).

The *MAT* genes determine the mating strategies of a fungal species (54), which include either heterothallism (self-incompatible), primary homothallism (self-compatible), or secondary homothallism (self-compatible) (50, 55). In heterothallic fungi, individuals possess either the *MAT1-1* or *MAT1-2* idiomorph in each haploid nucleus, and mating occurs only between partners of opposite mating types to complete the sexual life cycle (56). In contrast, primary homothallic species typically possess both mating

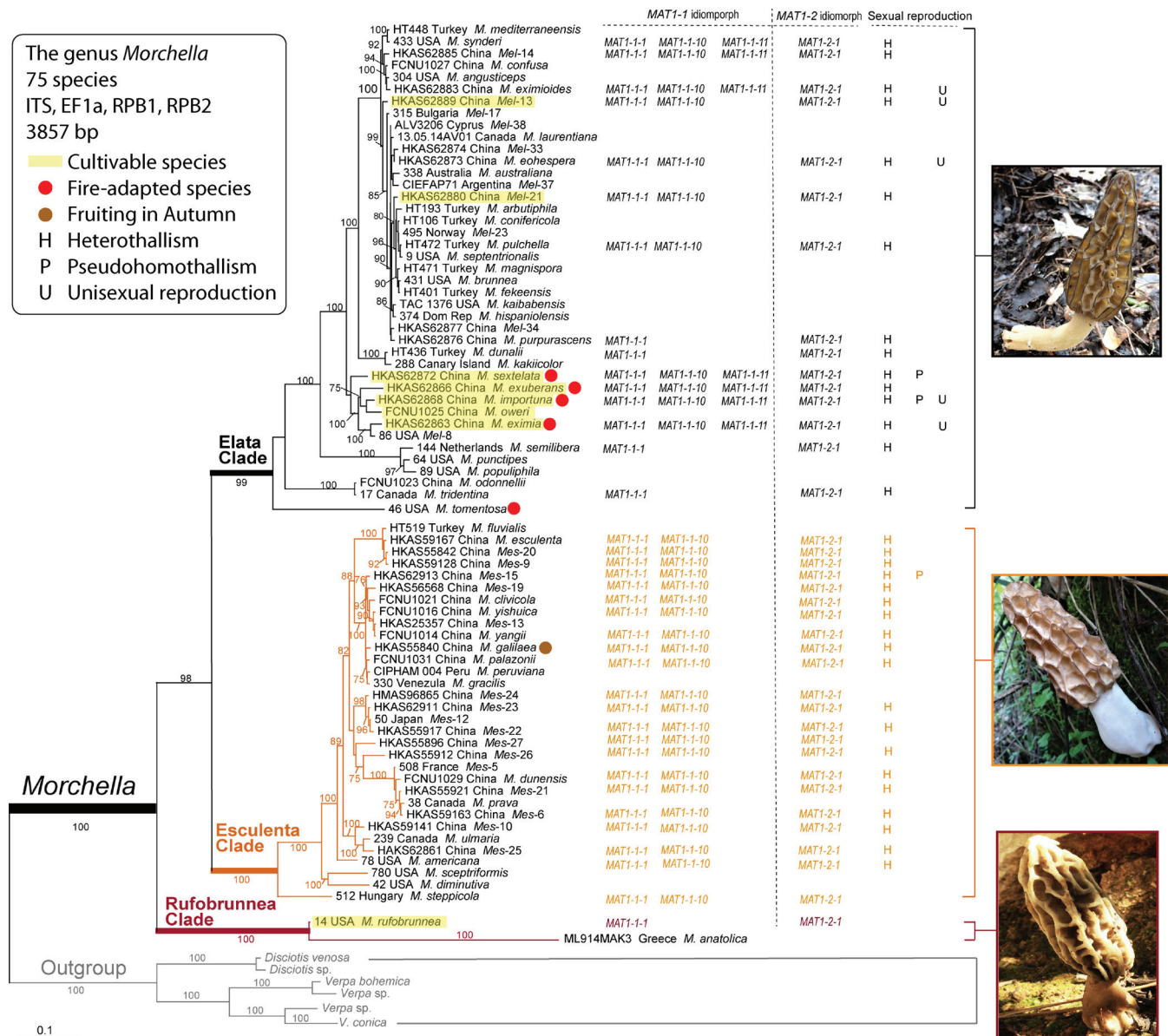


FIG 1 Maximum likelihood (ML) phylogenetic analysis of 75 species in the genus *Morchella* based on a four-gene data set (ITS plus *EF1- α* plus *RPB1* plus *RPB2*), with *Verpa* and *Disciotis* as outgroups. The species *Morchella castanea*, *M. vulgaris*, and *Morchella* sp. *Mes-28* were not included in this analysis because only ITS sequences for them are available from GenBank. The ML analyses were run in PhyML 3.0 using the GTR+I+G model of molecular evolution. The numbers by the nodes represent branch support >75%. Mating-type genes detected and sexual reproduction modes known in the species of *Morchella* are listed on the right. (Photo of *M. rufobrunnea* of the Rufobrunnea clade courtesy of Michael Loizides, reproduced with permission.)

types in each haploid nucleus (57) and are thus able to complete the sexual cycle without seeking a mate. Two mating-type idiomorphs are fused in a single genome or unlinked in a single genome but at different genomic regions (44, 56, 58).

In secondary homothallic fungi, there are two types of mechanisms, which have been described as pseudohomothallism and mating-type switching. Pseudohomothallic species usually package nuclei of opposite mating types (either mating type in each haploid nucleus) within a single multinucleus spore and are fully able to undergo independent sexual reproduction (59, 60). Mating-type switching is a “cassette model,” in which each haploid individual expresses only one mating type, even though it carries the information of both mating types (61, 62). The “silent” copies of the alternate mating type lie in a region of the genome that is transcriptionally nonactive due to the modified chromatin structure and are used during asexual reproduction to change the mating type through the transposition of information from a silent cassette to an active site (50, 62). The HO gene, vital for

mating-type switching, encodes an endonuclease responsible for the initiation of switching by generating the double-stranded DNA break, which induces the gene conversion between *MAT1-1* and *MAT1-2* (63). For example, in *Saccharomyces cerevisiae*, with the active *MAT* locus that governs the mating type, there are two *MAT* cassettes, *HML* and *HMR*, in the genome, which are normally kept silent by a modified chromatin structure (64). During mitosis, the existing allele at the active *MAT* locus can be replaced with the opposite allele from one of the two silent *MAT* cassettes via gene conversion (64). Due to the ability of switching mating type, this mating strategy may appear to be outwardly homothallic, as both mating types can be produced from a single haploid individual (65).

The most recently reported and, to date, incompletely elucidated form of homothallism is unisexual reproduction, first described by Lin et al. (66), also termed monokaryotic, homokaryotic, or haploid fruiting (58, 67), same-sex mating (68), or single mating-type reproduction (62). In contrast to primary homothallism, unisexual reproduction describes the process by which a single isolate independently undergoes sexual reproduction despite having genes of only one mating type (69), with sexual processes such as meiosis and genome diploidization being involved (70, 71), similar to parthenogenesis (46, 72). Though unisexual reproduction is suggested to be derived from heterothallism via the mutation of genes involved in the initiation of sexual reproduction (69), the molecular mechanisms of unisexual reproduction are not yet fully elucidated.

In ascomycetes, *MAT* idiomorphs (*MAT1-1* and *MAT1-2*) include two primary *MAT* genes (*MAT1-1-1* and *MAT1-2-1*) and other secondary *MAT* genes, which include 11 other *MAT1-1* genes (*MAT1-1-2* to *MAT1-1-11* plus "*MAT1-1-10*," a later homonym of *MAT1-1-10*) and 11 other *MAT1-2* genes (*MAT1-2-2* to *MAT1-2-12*) reported from the *MAT* locus of various species (23, 73, 74). The two primary *MAT* genes, *MAT1-1-1* and *MAT1-2-1*, have been functionally characterized in many ascomycete fungi and are typically essential for successful sexual reproduction, although their precise functions may change with species (75). In *Podospora anserina*, *Aspergillus nidulans*, and *Neurospora crassa* (76–79), both genes are known to be essential for fertilization, but the *MAT1-1-1* gene is also essential for ascospore production in *P. anserina* (77).

The *MAT1-1-1* and *MAT1-2-1* genes encode highly conserved proteins for the alpha domain of *MAT1-1* and an HMG box domain of *MAT1-2*, respectively, which possess DNA binding domains (51, 72, 80). The *MAT* proteins act as transcription factors that regulate the expression of several hundred genes related to sex, such as those involved in mate recognition of *MAT1-1* and *MAT1-2* identities, the pheromone and receptor systems, the conversion of vegetative mycelia into sexual tissue, cellular differentiation, and meiosis (50, 76, 81–86). In heterothallic ascomycete fungi, mate recognition is initiated by cell-specific pheromone and receptor combinations (87, 88), regulated by the *MAT1-1-1* protein and the *MAT1-2-1* protein (69, 89). Mating-type-specific pheromone receptors are located on the cell surface and sense the reciprocal signals (50). In homothallic ascomycete fungi, which harbor both *MAT* genes, both pheromone precursors are usually expressed (90). However, the discovery of unisexual reproduction has overturned the general view that the expression of two primary *MAT* genes is indispensable for sexual reproduction in fungi (64). The pheromone expression and response pathway of unisexual reproduction remain unclear.

The secondary *MAT* genes, which are not highly conserved as the primary *MAT* genes, usually do not have typical functional domains and have only been partially characterized (57, 75). For example, the *MAT1-1-2* gene of *P. anserina* (91) and *MAT1-1-5* gene of *Sclerotinia sclerotiorum* (92) are essential for producing sexual ascospores, the *MAT1-1-3* gene of *P. anserina* is beneficial for determining the nuclear identity (91), and the *MAT1-2-4* gene of *S. sclerotiorum* (92) and *MAT1-2-10* gene of *Botrytis cinerea* (93) act on ascumatal development.

During the sexual process in ascomycete fungi, a key feature is that ploidy changes from diploid to haploid and back to diploid via meiosis and sporulation (45). Meiosis is integral to this process and increases diversity through recombination. Two key meiosis-

specific genes, *SPO11* and *DMC1*, are critical for meiosis (64). *SPO11*, which is highly conserved across eukaryotes (94), encodes the endonuclease that generates the double-strand DNA breaks that provoke meiotic recombination, and *DMC1*, which is important in the processing of double-strand DNA breaks for repair during homologous recombination, encodes a meiotic recombinase (45, 95). As the master regulator of meiosis, *IME1* is only active during meiotic events and encodes a transcription factor that activates the expression of early meiotic genes (63, 96). Other genes with a specific function during meiosis include *IME2*, *NDT80*, *RME1*, *SET3*, etc. (63, 96). These genes, *SPO11*, *DMC1*, *ME1*, *IME2*, *IME4*, *KAR4*, *NDT80*, *DIG1*, and others, are also involved in sporulation, and their deletion severely impairs or entirely disables sporulation (66, 97, 98).

MATING-TYPE STRUCTURES IN MORCHELLA

As a new focus of intense research in the fields of biology and cultivation of edible fungi, morels and their reproductive modes are of interest not only in the context of basic questions regarding their biology, but also for the improvement of their cultivation. Several studies have reported possible reproductive modes of morel species (26, 99–101), but structures of the mating-type genes, mating system, and sexual reproduction of true morels have only recently been confirmed through the availability of the genome sequences of the species *M. importuna*, *M. eximia*, *M. yangii*, *Morchella* sp. *Mes-15*, and *Morchella* sp. *Mes-21* in the genus *Morchella* (20–25).

With the draft genomes database of *M. eximia* and *Morchella* sp. *Mes-21*, Du et al. (20, 21) identified the α domain of *MAT1-1-1* and the MATA_HMG-box of *MAT1-2-1* from both species and then subsequently obtained the α domain and MATA_HMG-box from 34 other species in *Morchella* by performing PCR using specially designed primers. Based on the genomes of two *M. importuna* ascospore isolates with either mating type and the resultant primers, Chai et al. (22, 23) identified the *MAT* locus and its flanking regions of *M. importuna* and were able to obtain the *MAT* locus of *Morchella* sp. *Mes-20* as well as *MAT1-1-1* and *MAT1-2-1* genes of 15 other species. With the *MAT* locus and its flanking regions characterized in the genomes of *M. importuna* (24), the genome of one *MAT1-1* ascospore isolate from a yellow morel species was sequenced, with a size of approximately 56 Mb and with at least 14 chromosomes identified, by Liu et al. (25), in which the species was regarded as *M. crassipes*, based on the ITS sequence [FJ860052.1](#). However, our analysis indicated that [FJ860052.1](#) and the ITS sequence of the genome-sequenced yellow morel are identical to that of *Morchella* sp. *Mes-20*, and *M. yangii*, respectively. In this review, given the detailed analysis on the *MAT* locus and flanking regions of both *M. importuna* (22, 24) and *Morchella* sp. *Mes-20* (23; X.-H. Du, L. Liu, and K. Chen, unpublished data), we focus particular attention on the mating type structures in the two species. Information about mating-type genes in other species in *Morchella* is also discussed later, with detailed information given in Table 1.

The genome of single-ascospore strains of *M. importuna*, with either *MAT1-1* and *MAT1-2* present, is reported to be approximately 46 Mb (22) or 51 Mb (24). The number and characters of chromosomes in the genome of *M. importuna* remain unknown. Analyses of the *M. importuna* genomes revealed that the lengths of *MAT1-1* and *MAT1-2* idiomorphs are 10.5 and 6.7 kb, respectively (22). Detailed analysis of the structure of the *MAT* locus showed that the *MAT1-1* idiomorph contains *MAT1-1-1* and two further newly described *MAT* genes, *MAT1-1-10* and *MAT1-1-11*, which were first reported and named from *Morchella*, while *MAT1-2* contains only a single *MAT1-2-1* gene (22, 23) (Fig. 2A). The *MAT1-1-1* gene, with a size of 1,694 bp, contains two introns and a conserved α domain; the *MAT1-2-1* gene, at 1,197 bp in size, contains three introns and a conserved MATA_HMG-box. The *MAT1-1-10*, at 1,688 bp in size, contains four introns and encodes a hypothetical idiomorph-specific protein with 488 amino acids; the *MAT1-1-11* gene, at 1,825 bp in length, contains five introns and encodes an idiomorph-specific hypothetical protein with 512 amino acids (22, 23) (Fig. 2A). The *MAT* genes, including *MAT1-1-1*, *MAT1-1-10*, *MAT1-1-11*, and *MAT1-2-1*, are constitutively expressed in *M. importuna* under laboratory conditions (22, 23). However, no further

TABLE 1 Known sexual reproduction modes and mating-type genes of species in *Morchella* and the approximate number of nuclei in their ascospores

Clades	Species	MAT1-1-1	MAT1-1-10	MAT1-1-11	MAT1-2-1	Heterothallism	Pseudohomothallism	Unisexual reproduction	No. of nuclei per ascospore
Rufobrunnea clade	<i>M. rufobrunnea</i>	√ ^a	U ^b	U	√	U	U	U	U
Elata clade	<i>M. tridentina</i>	√	×	×	√	√	U	U	N ≥ 10
	<i>M. semilibera</i>	√	×	×	√	√	U	U	N ≥ 6
	<i>M. sextelata</i>	√	√	√	√	√	U	U	N ≥ 4
	<i>M. eximia</i>	√	√	√	√	√	U	√	N ≥ 6
	<i>M. exuberans</i>	√	√	√	√	√	U	U	N ≥ 6
	<i>M. importuna</i>	√	√	√	√	√	U	√	N ≥ 6
	<i>Morchella</i> sp. Mel-13	√	√	×	√	√	U	√	N ≥ 10
	<i>Morchella</i> sp. Mel-14	√	√	√	√	√	U	U	N ≥ 6
	<i>M. eximoides</i>	√	√	√	√	√	U	√	N ≥ 6
	<i>M. eohespera</i>	√	√	×	√	√	U	√	N ≥ 10
	<i>M. purpurascens</i>	√	×	×	√	√	U	U	N ≥ 6
	<i>Morchella</i> sp. Mel-21	√	√	×	√	√	U	U	N ≥ 6
	<i>M. dunalli</i>	√	×	×	√	√	U	U	N ≥ 6
	<i>M. pulchella</i>	√	√	×	√	√	U	U	N ≥ 6
	<i>M. snyderi</i>	√	√	×	√	√	U	U	N ≥ 6
	<i>Morchella</i> sp. Mes-6	√	√	√ ^d	√	√	U	U	U
	<i>Morchella</i> sp. Mes-9	√	√	√	√	√	U	U	N = 2/4/6/8/≥10
	<i>Morchella</i> sp. Mes-10	√	√	√	√	√	U	U	N = 2/4/6/8/≥10
	<i>Morchella</i> sp. Mes-15	√	√	√	√	√	U	U	N ≥ 10
	<i>Morchella</i> sp. Mes-19	√	√	√	√	√	U	√	N ≥ 10
<i>Morchella</i> sp. Mes-20	√	√	√	√	√	U	√	N = 2/4/6/8/≥10	
<i>Morchella</i> sp. Mes-21	√	√	√	√	√	U	U	N = 2/4/6/8/≥10	
<i>Morchella</i> sp. Mes-22	√	√	√	√	√	U	U	N = 2/4/6/8/≥10	
<i>Morchella</i> sp. Mes-23	√	√	√	√	√	U	U	N = 2/4/6/8/≥10	
<i>Morchella</i> sp. Mes-24	√	√	√	√	√	U	U	N = 2/4/6/8/≥10	
<i>Morchella</i> sp. Mes-25	√	√	√	√	√	U	U	U	
<i>Morchella</i> sp. Mes-26	√	√	√	√	√	U	U	N ≥ 10	
<i>Morchella</i> sp. Mes-27	√	√	√	√	√	U	U	N ≥ 10	
<i>M. americana</i>	√	√	√	√	√	U	U	U	
<i>M. clivicola</i>	√	√	√	√	√	U	U	U	
<i>M. dunensis</i>	√	√	√	√	√	U	U	U	
<i>M. esculenta</i>	√	√	√	√	√	U	U	U	
<i>M. galilaea</i>	√	√	√	√	√	U	U	U	
<i>M. palazonii</i>	√	√	√	√	√	U	U	U	
<i>M. steppicola</i>	√	√	√	√	√	U	U	U	
<i>M. yangii</i>	√	√	√	√	√	U	U	U	
<i>M. yishuica</i>	√	√	√	√	√	U	U	U	

^a√, gene or sexual reproduction mode was reported in the species.
^bU, gene, sexual reproduction mode, or number of nuclei per ascospore was not reported in the species so far.
^c×, gene was not detected in the species by performing PCR amplification.
^d√, analysis of MAT1-1-11 was not applied in the species.

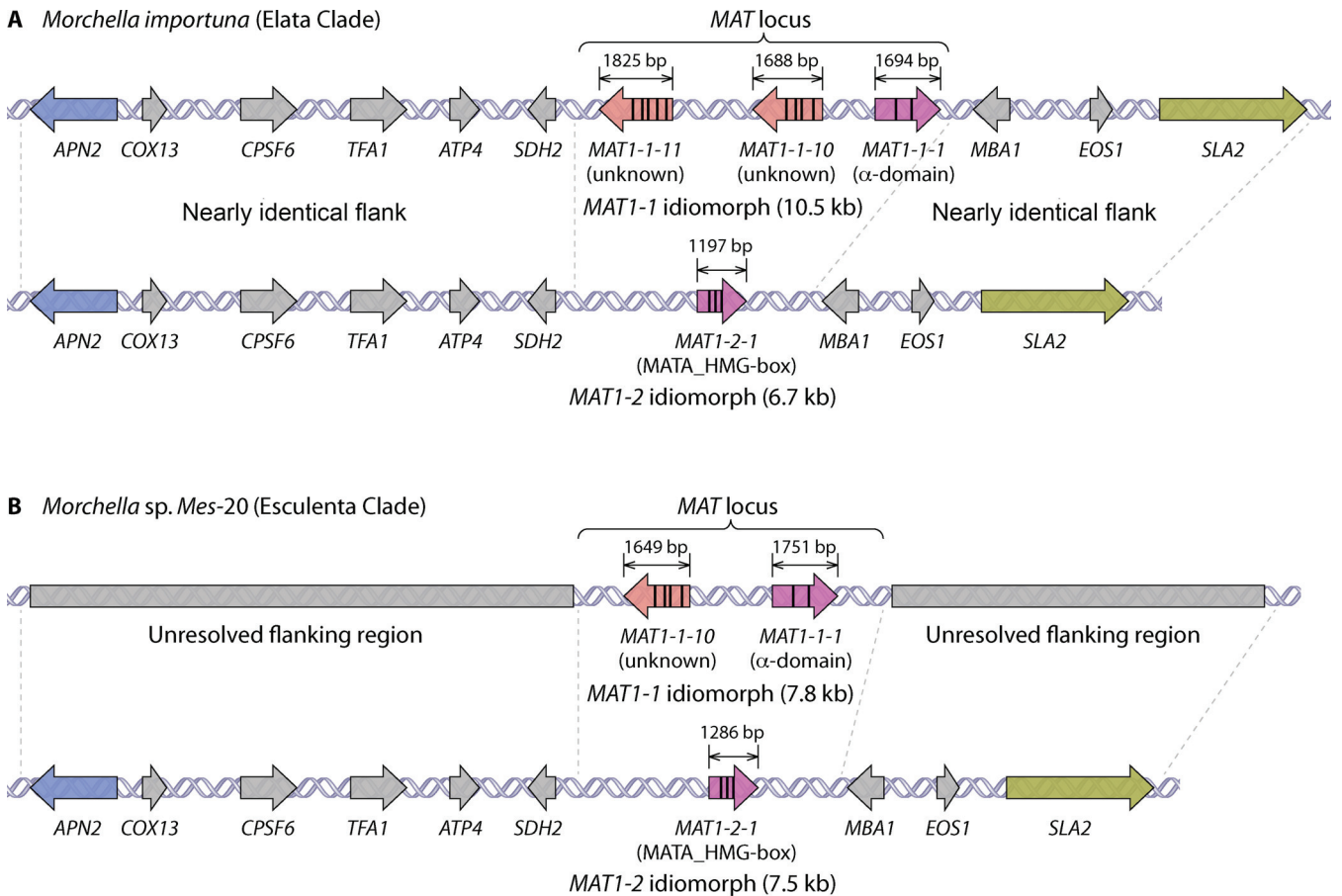


FIG 2 Structure of the *MAT* locus of *Morchella importuna* (Elata clade) (A) and *Morchella* sp. *Mes-20* (Esculenta clade) (B). Introns are represented inside the *MAT* genes with vertical black lines. At the *MAT* locus, either a *MAT1-1* or *MAT1-2* idiomorph is present, containing at least a *MAT1-1-1* α -domain gene or a *MAT1-2-1* MATA_HMG-box gene, respectively. In addition, further genes are present in the *MAT1-1* idiomorph: *MAT1-1-10* and *MAT1-1-11* in *M. importuna* (A) and *MAT1-1-10* in *Morchella* sp. *Mes-20* (B). The genes flanking the *MAT* idiomorphs are fairly well conserved. The flanking region of the *MAT1-1* idiomorph of *Morchella* sp. *Mes-20* has not yet been revealed. *APN2* encodes an abasic endonuclease/DNA lyase; *COX13* encodes the cytochrome *c* oxidase subunit VIa; *CPSF6* encodes cleavage and polyadenylation specificity factor subunit 6; *TFA1* encodes transcription initiation factor IIE subunit alpha; *ATP4* encodes mitochondrial membrane ATP synthase subunit 4; *SDH2* encodes succinate dehydrogenase (ubiquinone) iron-sulfur subunit; *MBA1* encodes mitochondrial inner membrane-associated mitoribosome receptor; *EOS1* encodes *N*-glycosylation protein; and *SLA2* encodes a protein that binds to cortical patch actin. Distances and sizes are not drawn to scale. Mating-type structures from *M. importuna* and *Morchella* sp. *Mes-20* were summarized from references 20–24 and our unpublished data.

progress has been made on elucidating the molecular biological function of the *MAT1-1-1*, *MAT1-2-1*, *MAT1-1-10*, and *MAT1-1-11* genes in *M. importuna* (22). It is worth noting that the *MAT1-1-10* gene was not found in the genome of *M. importuna* by Liu et al. (24). We used the primers designed by Chai et al. (22) to perform PCR and did detect the *MAT1-1-10* gene from samples of *M. importuna*.

The regions flanking the two idiomorphs are highly conserved in *M. importuna*. The flanking sequences of the *MAT1-1* idiomorph harbor the same genes in the same syntenic orientation as those of the *MAT1-2* idiomorph (22, 24). However, the pattern of flanking genes and the gene names are slightly different between Chai et al. (22) and Liu et al. (24). An extra flanking gene, *EOS1*, was given by Liu et al. (24) but not by Chai et al. (22). Analysis of our unpublished genomic data indicated the gene *EOS1* does exist in the flanking regions of *MAT* locus in *M. importuna*. Moreover, the genes encoding the same protein are annotated with different names by Chai et al. (22) and Liu et al. (24), namely, *atp-3/ATPs4*, and *Mba1/Mab1*. By searching for the information in UniProt, a freely accessible database on proteins and their functions, the names of two genes should be *ATP4* and *MBA1*, respectively. Taking the total information into account, the genes flanking the *MAT* locus of *M. importuna* are shown in Fig. 2A.

The genome of *Morchella* sp. *Mes-20* generated from one *MAT1-2* ascospore isolate

is haploid, and the size is approximately 48 Mb (X.-H. Du, L. Liu, and K. Chen, unpublished data). The *MAT1-1* idiomorph is 7.8 kb in length and harbors the *MAT1-1-1* and *MAT1-1-10* genes, while the *MAT1-2* idiomorph is 7.5 kb in length, containing only the *MAT1-2-1* gene (23). The *MAT1-1-1* gene, which has a size of 1,751 bp, contains two introns and a conserved α domain; the *MAT1-2-1* gene, which is 1,286 bp in size, contains three introns and a conserved MATA_HMG-box. The *MAT1-1-10*, with a size of 1,649 bp, contains four introns and encodes a hypothetical idiomorph-specific protein with 476 amino acids (23) (Fig. 2B). The *MAT1-1-1*, *MAT1-2-1*, and *MAT1-1-10* genes are expressed in *Morchella* sp. *Mes-20* under laboratory conditions; however, their molecular functions remain unknown (23). The flanking sequences of the *MAT1-2* idiomorph of *Morchella* sp. *Mes-20* are highly conserved and harbor the same genes as those of the *MAT1-1* and *MAT1-2* idiomorphs of *M. importuna* (22–24; X.-H. Du, L. Liu, and K. Chen, unpublished data), with the pattern shown in Fig. 2B. The flanking regions of the *MAT1-1* idiomorph in *Morchella* sp. *Mes-20* are not confirmed yet. Considering the conservative character of this area, we presume the flanking regions of its *MAT1-1* idiomorph are likely to be the same as those of its *MAT1-2* idiomorph.

The size and arrangement of the *MAT1-2* idiomorph (7.5 kb) of *Morchella* sp. *Mes-20* is similar to that of *M. importuna* (6.7 kb), while the size of the *MAT1-1* idiomorph is truncated in *Morchella* sp. *Mes-20* (7.8 kb) due to a missing *MAT1-1-11* gene compared with that of *M. importuna* (10.5 kb) (23). Also, 66.5%, 72.1%, and 71.1% of the *MAT1-2-1*, *MAT1-1-1*, and *MAT1-1-10* genes of *Morchella* sp. *Mes-20*, respectively, are identical to those of *M. importuna* (23). In *Morchella*, the *MAT1-2* idiomorph is less complex than that of the *MAT1-1* idiomorph.

In filamentous ascomycetes, the *MAT* idiomorphs usually vary in size from 1.2 to 5.7 kb (56), but in certain taxonomic groups, the size of the *MAT* locus can be up to 6 kb or larger due to the inclusion of additional genes (102). The *MAT1-1* and *MAT1-2* idiomorphs of *Coccidioides* are 8.1 and 9 kb in length, respectively, and were once reported to be the largest for ascomycetes (103). However, the *MAT1-1* idiomorph of *M. importuna*, reported to be 10.5 kb in size, is much larger than that of *Coccidioides* and may well be the largest *MAT1-1* idiomorph in filamentous ascomycetes, whereas the *MAT1-2* idiomorphs of either *M. importuna* (6.7 kb) or *Morchella* sp. *Mes-20* (7.5 kb) are both smaller than that of *Coccidioides* (22, 23, 103).

The *MAT* locus normally occupies a similar chromosomal position between homologs of *APN2* and *SLA2* in many filamentous ascomycetes (57, 104). In Sordariomycetes, homologs of *APN2* and *SLA2* are adjacent to each side of the *MAT* locus in almost all species, with an interruption only in a few species (104). In *Morchella*, the 5' flanking regions of both *MAT* idiomorphs contain *APN2*, *COX13*, and four other predicted genes, and the 3' flanking region houses *SLA2* and two other predicted genes. However, the molecular functions of the flanking genes of the *MAT1-1* and *MAT1-2* idiomorphs in *M. importuna* and *Morchella* sp. *Mes-20* are as yet unknown.

In *Morchella*, the *MAT1-1-10* and *MAT1-1-11* genes were first reported for the Elata clade in the *MAT1-1* idiomorph of *M. importuna*, and the *MAT1-1-10* gene was first reported in the Esculenta clade in the *MAT1-1* idiomorph of *Morchella* sp. *Mes-20* (22, 23). To confirm whether these new genes exist in other species of *Morchella*, we performed a facile PCR test to rapidly assess presence or absence of these genes in samples of 14 other species in the Elata clade and 21 species in the Esculenta clade, using the primers designed by Chai et al. (23) and X.-H. Du, H. Li, and B. Yuan (unpublished data). Results showed that *MAT1-1-10* exists in all the tested species from the Esculenta clade. In Elata clade, besides *M. importuna*, both the *MAT1-1-10* and *MAT1-1-11* genes were also found in an additional six species but partially failed to be amplified in other species (Table 1) (X.-H. Du, H. Li, and B. Yuan, unpublished data). Future genomic studies should uncover whether these species harbor *MAT1-1-11* (and *MAT1-1-10*). To date, of the 38 species studied from the Rufobrunnea, Elata, and Esculenta clades of *Morchella*, all are confirmed to have *MAT1-1-1* and *MAT1-2-1* genes, while seven species from the Elata clade also harbor both *MAT1-1-10* and *MAT1-1-11*, and 22 species from the Esculenta clade contain *MAT1-1-10* as well. The

mating-type genes currently obtained from each species are listed in Table 1 and are shown in Fig. 1. The structures of both idiomorphs and their flanking regions of *M. importuna* and *Morchella* sp. *Mes-20* are summarized in Fig. 2.

EVOLUTION OF MATING-TYPE GENES IN MORCHELLA

Although sexual reproduction in fungi is associated with dynamic evolution of sexual behaviors and mating-type-determining mechanisms, it may influence important evolutionary and ecological processes, such as speciation and adaptation (105, 106). Accordingly, the phylogenetic relationships between the *MAT1-1-1* and *MAT1-2-1* genes from different species of *Morchella* were examined to elucidate the evolution of mating-type genes in *Morchella* (20, 21, 23). In ascomycetes, mating-type genes have evolved more rapidly than other protein-coding genes (107, 108). That the interspecific nucleotide diversity of *MAT1-1-1* and *MAT1-2-1* sequences is higher than the intraspecific nucleotide diversity suggests that *MAT* genes evolve rapidly in *Morchella* (20, 21, 23). This is also consistent with the strong purifying selection against deleterious mutations in *MAT* genes (46, 109).

The *MAT* genealogies and species phylogeny of the Elata clade share similar topologies, and the two *MAT* genes were recommended as good candidate markers for phylogenetic inferences in this clade (20, 23). For the Esculenta clade, Du et al. (21) reported that the *MAT* genealogies, generated from the alpha domain of *MAT1-1* and an HMG box domain of *MAT1-2*, and a species phylogeny of 22 yellow morel species had different topologies. The *MAT* genealogies reported by Du et al. (21) resolved most of the species, indicating their potential usefulness as phylogenetic markers for species delimitation. However, several species could not be distinguished. Chai et al. (23) compared the genealogies of *MAT1-1-1* and *MAT1-2-1* with a phylogeny of nine species generated from the combined ITS plus 28S plus *RPB1* plus *RPB2* plus *EF1- α* data set. The authors reported that either *MAT* gene could be used to distinguish each of the nine species, although the trees had different topologies. The highly conserved HMG and α -box domains of the mating-type genes found in *Morchella* (23) might partially contribute to the unresolved phylogeny of several species reported by Du et al. (21). However, the potential introgression of the two *MAT* idiomorphs into a species from a single ancestral source could not be excluded, and a more extensive analysis incorporating additional samples is required to resolve this question.

The evolution of *MAT* genes is commonly regarded as a source of reproductive isolation during speciation (80). Reproductive isolation acts to preserve the genetic integrity of species and prevents the introduction of “foreign” DNA (110). However, the phylogenies of *MAT* idiomorphs in the genus *Morchella* presented by Du et al. (20, 21) and Chai et al. (23) highlight a few well-supported topological discordances from the species trees which can be taken as an indication of potential interspecific hybridization.

In fungi, interspecific hybridization can be achieved by sexual mating or by fusion of vegetative structures (111) and acts as a major factor affecting speciation and adaptation (112, 113). In *Morchella*, multiple potential interspecific hybridization events have been reported between species within the Elata clade (26–28) or within the Esculenta clade (21), as well as between species of the aforementioned two clades (X.-H. Du and K. Chen, unpublished data). These interspecific hybridization events in *Morchella* suggest that the phylogenetic divergence of morels might have preceded the development of reproductive barriers, as has also been proposed for other fungi (114–116). Taken together, these works highlight the complex evolutionary trajectories of the reproductive genes of *Morchella*, which are important for the understanding of speciation and reproductive behavior in this genus.

Inbreeding has also been indicated in *Morchella* in several studies (26, 99, 100, 117). Typically, in heterothallic species, heterozygosity can be identified when the genetic diversity of fruiting bodies is evaluated using codominant markers because of the existence of two different parental genotypes. However, little heterozygosity has been reported from *Morchella* except the *MAT* locus, which indeed indicates the occurrence

of frequent inbreeding (26, 99, 100, 117). Although heterozygosity in *M. importuna*, *M. sextelata*, *M. eximia*, *M. exuberans*, *Mel-13*, and *Mel-21* was reported using 22 genetic markers (simple sequence repeats [SSRs]), the observed intraspecific heterozygosity was lower than expected, and the discrepancy was attributed to forces such as inbreeding (27). In this case, the parents may have been closely related (inbred), which would potentially explain why little marker segregation was found in *Morchella*. In heterothallic species, the recognition and response of gametes of opposite mating types is regulated by pheromones and pheromone receptors produced in a mating-type-specific manner (54). This probably means that parental fungi that are physically close and therefore more likely to be related to each other may more easily sense each other and receive pheromone signals to mate than more geographically distant individuals, ultimately resulting in the prevalence of inbreeding in morels. This is similar to the results from works in *Tuber melanosporum*, which suggest that two mating partners most probably have to be located sufficiently close to allow sensing each other for mating (118). However, the exact mechanisms that underpin the frequent inbreeding of morel species remain unknown.

MATING SYSTEMS IN MORCHELLA

Sex involving two mating partners, males and females with clear morphological differences, is compulsory in some eukaryotes. However, in fungi, the two mating partners are morphologically indistinguishable for most of their life cycles (51, 102). Mating types are used to define the sexual identity of a fungal individual, according to its ability to recognize and mate with different sexual cell types, and then to determine the mode of sexual reproduction (45, 56).

The availability of the complete or draft genome sequences has created the opportunity to unveil the structure of the *MAT* locus and mating strategies in the genus *Morchella*. Knowledge of *MAT* genes in *M. eximia* (20), *M. importuna* (22, 24), *Morchella* sp. *Mes-21* (21), and *Morchella* sp. *Mes-20* (23) made it possible to develop universal primers that can be used for amplification of *MAT* genes and sequencing of these genes from other species of this genus.

To date, in *Morchella*, *MAT1-1-1* and *MAT1-2-1* genes have been successfully amplified from one species of the *Rufobrunnea* clade (23), 15 species of the *Elata* clade (20, 22, 24), and 22 species of the *Esculenta* clade (21, 23) (Fig. 1, Table 1). Single-spore analyses were conducted in all of the species except *Morchella* sp. *Mes-24*, *Morchella* sp. *Mes-27*, and *M. rufobrunnea*, and the results revealed that the ascospores produced by each species harbor a single idiomorph with nearly equal ratios of both mating types (20–25). The results support the notion that these species are self-sterile and have a heterothallic life style (20–25). Notably, the sexual reproductive mode of *M. rufobrunnea*, a basal species in *Morchella*, remains a mystery. This needs to be investigated further to get a better understanding of reproductive modes and its evolution in *Morchella*.

Remarkably, as well as the heterothallism mentioned above, it has also been discovered that unisexual reproduction, the most recent known homothallism, can also, if rarely, occur in *Morchella* (20). In *M. eximia*, *M. importuna*, *Morchella* sp. *Mel-13*, *M. eximioides*, and *M. eohespera*, only one idiomorph was detected in the individual fruiting bodies, which suggests that unisexual reproduction (haploid fruiting) does occur in these species, without the involvement of an opposite mating type (20). That means some species in *Morchella* have two sexual reproduction modes, heterothallism and unisexual reproduction (homothallism). Du et al. (20) did not observe any ascospores in the hymenium at maturity in those ascocarps produced by unisexual reproduction, indicating a possible failure to complete the life cycle. Sterile fruiting bodies and a lack of ascospores have also been reported in *Cordyceps militaris* (119, 120). However, one or two asci with ascospores, rarely up to 13, were observed to be produced in mtA perithecia in *Sordaria brevicollis* (121), even though the majority were empty.

Interestingly, pseudohomothallism has recently been reported in the species *M. sextelata*, *M. importuna*, and *Morchella* sp. *Mes-15*, in which single ascospores harboring

both *MAT1-1* and *MAT1-2* genes were found (122). One wild and one cultivated fruiting body of *M. importuna* were analyzed, and heterokaryon ascospores were only detected in the wild one, with a ratio of 12.1% (four heterokaryon ascospores detected from 33 investigated ascospores). The ratios of heterokaryon to total ascospores were approximately 10% and 1.2% in *M. sextelata* and *Morchella* sp. *Mes-15*, respectively, when single wild ascocarps were analyzed (122). The biological characters and ontogeny of the heterokaryon ascospores in *Morchella* remain unknown.

To date, three kinds of sexual reproductive modes have been found in *Morchella*: heterothallism, unisexual reproduction (homothallism), and pseudohomothallism. Mixed mating systems do exist in several species of *Morchella*, and heterothallism is the dominant reproductive mode. To date, *M. importuna* is the only species in *Morchella* having all three sexual reproductive modes known from the genus, and it is also the species which is most widely cultivated in China. The known sexual reproductive modes of species in *Morchella* are listed in Table 1. It is anticipated that more species in *Morchella* having mixed sexual reproduction modes could be found in the future.

DOMINANT HETERTHALLISM WITH A PREVALENT HAPLOID LIFESTYLE

Separate sexes are not known in fungi, and most filamentous heterothallic ascomycetes mate in a hermaphroditic fashion, where the male and female roles are not associated with the *MAT* locus (*mat A/MAT1-1* or *mat a/MAT1-2*), and the sexual identity of a fungal individual is defined according to its ability to recognize and mate with different sexual cell types (56, 123). Haploid strains are hermaphroditic and self-sterile, mating by donation or acceptance of nuclei from other individuals with the opposite mating type (124, 125).

In *Morchella*, the genetic material in the ascocarps exhibits maternal and paternal partitioning, but the sexual identity is not genetically defined. The predominant partner (mating type) in the ascocarps of *Morchella* is usually defined as maternal or female, and the opposite mating type as paternal or male, as used in truffles (126–128). By defining the stipe as representing the sterile portion and the hymenium as representing the fertile portion, Du et al. (20) described three types of *MAT* segregation in *Morchella* ascocarps, namely, types I to III (Fig. 3).

In type I (Fig. 3A), the hymenium as well as the corresponding stipe of certain cultivated morel ascocarps from the species *M. importuna*, *M. sextelata*, and *M. eximia* harbor both *MAT* genes, unlike wild morels (as described below). Du et al. (20) speculated that this might be related to the available nutrition at the cultivated sites. However, given the recently observed pseudohomothallism in *Morchella* (122), especially in *M. importuna* and *M. sextelata*, we presume that this type of fruiting body is likely to develop from the heterokaryotic ascospore and be constructed from heterokaryotic mycelia and tissue, with both mating types found in the hymenium and stipe.

Type II (Fig. 3A) is the dominant distribution type of *MAT* genes observed in wild morel ascocarps. In this type, the hymenium harbors both *MAT* genes, but only a single mating type (either *MAT1-1* or *MAT1-2*) is exclusively amplified in the corresponding stipe. As in other ascomycetes, including the truffle species, this implies that a haploid life cycle is prevalent (129, 130). The one mating type (either *MAT1-1* or *MAT1-2*) is likely to contribute to the formation of the main structure of the ascocarp (the stipe and the infertile tissues of the hymenium) (Fig. 3A and B), and is defined as maternal, behaving as a “maternal” partner or a female gamete during mating and growth. The other mating type, which is only detected in the hymenium (ascospore pools), is likely to have functioned as a “paternal” partner (the male gamete) via contribution to fertilization. A haploid morel strain may be hermaphroditic, with the ability to differentiate into maternal and paternal sexual structures, but the sexual cycle is initiated only when one mating type is fertilized by cells of a different mating type, as reported in *N. crassa* (124, 125). In heterothallic ascomycetes, hermaphroditism is common and expected in most species and supposedly represents the ancestral state (123, 131).

In filamentous fungi, heterokaryons could be formed by the fusion of somatic cells from different isolates, and somatic nonself recognition is controlled by vegetative

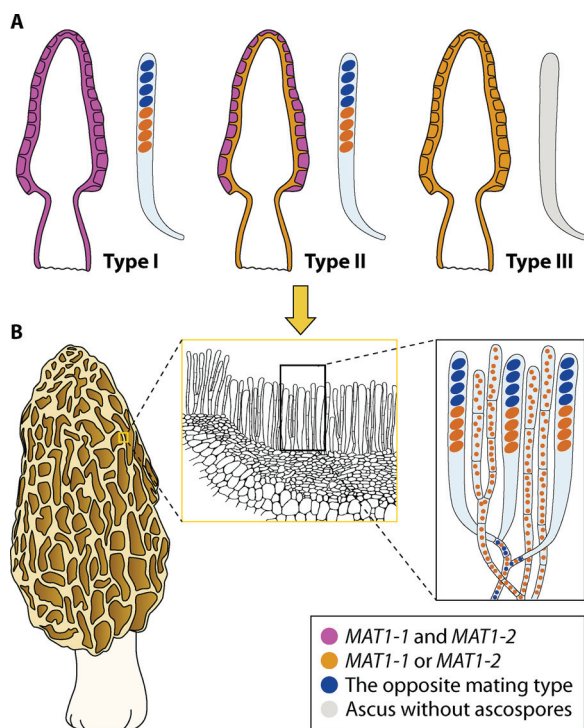


FIG 3 Schematic illustration of distribution of mating types in mature ascomata of morels. (A) Three recognized types: I (both mating types equally distributed and present in the hymenium and stipe), II (the dominant type, *MAT1-1* or *MAT1-2* acting female, and opposite as male, with both mating types present in the hymenium while either of them is present in the stipe), and III (only one *MAT* idiomorph present in the ascomata, no mating, and ascospores not formed). (B) Distribution of mating types in the hymenium of the type II morels.

incompatibility (78, 132–134). Recognition of a dominant haploid life cycle highlights the absence of heterokaryons during most stages of the morel life cycle, hinting at the existence of a system of vegetative incompatibility in *Morchella*. In ascomycetes, genetic nonself recognition systems usually prevent the formation of anastomosis between strains, likely at the perfusion level, which maintains the genetic integrity of each strain and protects resources within hyphae from exploitation by nonkin individuals during vegetative growth (132). However, it is not clear which genes play a role in vegetative incompatibility in *Morchella*, and the factors that regulate the transition from the vegetative to reproductive stage await to be uncovered.

Finally, in type III (Fig. 3A), only one idiomorph is detected in the fruiting body. This type of ascomata is rare and develops through unisexual reproduction from a haploid individual with only one mating type.

SPATIAL COMPETITION AND SKEWED DISTRIBUTION OF MATING TYPES

In some fungi, mating type genes not only drive sexual reproduction but also influence other functions, such as pathogenicity (135), virulence (54, 136), cellular processes (137), and cell wall integrity (138). These correlations were reported to likely cause the patchy and imbalanced distribution of two mating types at natural sites in some species, such as *C. militaris* (120), *Cryptococcus neoformans* (121), and *T. melanosporum* (128, 139). Even in species that reproduce asexually, an imbalanced distribution of mating types was readily observed, such as in *Teratosphaeria destructans* (140).

As mentioned in the section above, there are three types of ascocarps with different *MAT* distributions known from *Morchella* (20). To investigate the distribution of mating types in different populations of the same species, we focus here on the dominant type II ascocarps. In type II, the maternal partner (mating type) contributes to most parts of the ascocarps (the stipe and the infertile tissue of hymenium) (20). The stipe,

haploid with either mating type, has been chosen to represent the maternal tissue and can be monitored by *MAT* genotyping (20). *MAT1-1* was found to serve as the maternal partner overwhelmingly more often than *MAT1-2*, appearing in almost all investigated species of *Morchella* (20). In *M. eximoides*, a ratio of being maternal of nearly 6:1 for *MAT1-1* to *MAT1-2* was reported (20). Furthermore, an unexpectedly large imbalance was also reported from individual populations of *M. eohespera* and *Morchella* sp. *Mel-13*, in which the maternal partners of all investigated ascocarps were *MAT1-1* (20). Apparently, *MAT1-1* carries a strong competitive advantage acting as the maternal partner, and the frequency of its being so is skewed and unbalanced toward it. The observations suggest that *MAT1-1* tends more to serve a female sexual role than *MAT1-2*, and a strong sexual selection on *MAT1-1* exists in *Morchella*. Although separate sexes (male and female genders) are not known in fungi (123), these findings would suggest the mating types of *Morchella* are seemingly more like “sexes” than the typical (hermaphroditic) mating types.

The strong mating type competition and predominance of a single mating type in morels may reduce the opportunity for successful mating in some cases and cause inhibition of growth. It has been reported that the divergence of ecological, physiological, pathogenic, or other functional aspects of isolates having opposite mating types may affect the spatial distribution of some fungi (72, 135, 141, 142). However, the reasons underlying the skewed spatial distribution of opposite mating types in *Morchella* are unknown. In *Morchella*, *MAT1-2-1* shows higher genetic diversity than *MAT1-1-1* (20, 21, 23), which is likely related to potential competition and sexual selection.

IMPACT OF MATING TYPE ON THE ASEQUAL STAGE AND CONIDIAL PRODUCTION IN MORCHELLA

Asexual reproduction is an alternative to sexual reproduction and occurs in the majority of fungi, although the evolutionary forces governing the relationship between asexual and sexual reproductions are not well known. In *Morchella*, the mitospore stage (conidia) was first observed by Molliard (143, 144) and then repeatedly reported for other morel species at natural or cultivation sites (145–151). Alvarado-Castillo et al. (152) first reported the formation of chlamydospores in morels. Recently, Yuan et al. (153) successfully induced conidiation (Fig. 4A) in single-ascospore cultures of either mating type and in cross-mating cultures with opposite mating types of *M. sextelata*. They also observed the germination of conidia (Fig. 4A3 to A5) and chlamydospore formation from conidial hyphae (Fig. 4B1 to B5). These results support the notion that *Morchella* species produce two kinds of mitospores, conidia and chlamydospores.

Intriguingly, Yuan et al. (153) demonstrated that a vegetative mycelium (either *MAT1-1* or *MAT1-2* mycelium) formed by germinating ascospores could produce conidia, which supports the suggestion by Volk and Leonard (151) that conidia may be formed from vegetative mycelia as shown in their diagram. Hence, the ability of either *MAT1-1* or *MAT1-2* mycelium-producing conidia indicates, in turn, that the mating type of conidia could be *MAT1-1* or *MAT1-2*. In addition, the development of conidiophores in cross-mating cultures (*MAT1-1*:*MAT1-2*) was observed to occur earlier and to result in the production of more conidia than was observed in single-spore (*MAT1-1* or *MAT1-2*) cultures (153). This suggests that cross-mating cultures produce some specific compounds that may stimulate conidiation. The asexual stage and conidial production in morels are most likely to be impacted and regulated in a mating-type-specific manner. In *Ulocladium botrytis* (154) and *Penicillium chrysogenum* (155), *MAT* genes were demonstrated to play essential roles in different aspects of conidiation, such as conidial size and numbers.

Conidia are commonly observed in both wild and cultivated morels but, at first, were not observed to germinate or initiate a new colony, and were once thought to function as spermatia rather than reproductive propagules (147, 156–159). Yuan et al. (153) first observed the germination of conidia and their subsequent development into mycelia in *Morchella* and also demonstrated that conidia are able to act as reproductive propagules rather than only as spermatia. Since a morel strain of either mating type can be

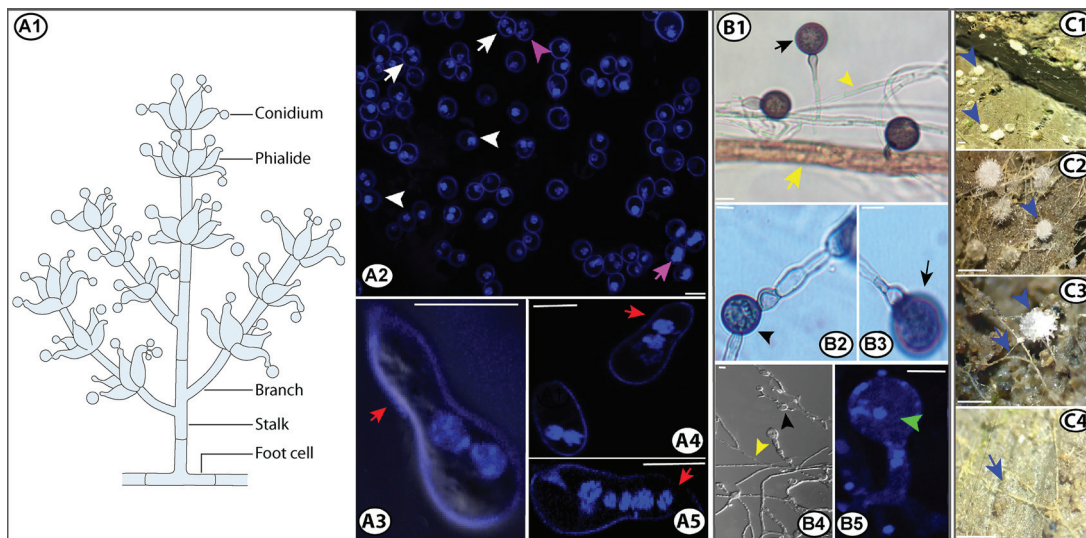


FIG 4 Schematic diagram of a conidiophore from *Morchella*, conidial nuclei, and conidial germination (A), chlamydospore formation from conidial hyphae (B), and primordia of morels and probable conidial hyphae at cultivation sites (C). Uninucleate (white arrowheads, A2), binucleate (white arrows, A2), and trinucleate conidia (purple arrowhead, A2). Nucleus mitosis in detached conidia (purple arrow, A2). Conidial germination (red arrows, A3 to A5). Terminal chlamydospores (black arrows, B1 and B3). Intercalary chlamydospores (black arrowheads, B2 and B4). Nuclei of chlamydospores (green arrowhead, B5). Much narrower conidial hyphae (yellow arrowheads, B1 and B4) than other hyphae (yellow arrow, B1). Primordia from morels (blue arrowheads, C1 to C3). Putative conidial hyphae at cultivation sites (blue arrows, C3 to C4). Bars, 5 μ m (A and B) or 0.2 mm (C). (Panels A and B are modified from reference 153 with permission from the British Mycological Society.)

hermaphroditic and have the potential to form “maternal” and “paternal” sexual structures (20), we presume that morel conidia might act as fertilizing agents (spermatia) or germinate to act as a hermaphroditic and facultative mycelium performing the function of fertilizing agents or reproductive propagules. Although hyphae derived from conidia were observed at cultivation sites and around primordia (Fig. 4C), there is still no direct evidence that shows conidia or hyphae derived from them acting as fertilizing agents. On the other hand, it remains an open question whether conidia are indispensable for morel fruiting, considering that some morel fruiting bodies are seemingly produced without conidial formation observed outwardly at the cultivation sites.

HYPOTHETICAL LIFE CYCLE OF MORCHELLA

Because of the high economic value of morels, illumination of the life cycle of *Morchella* species is crucial to understand their biology and advance morel cultivation technology. Over the past few decades, investigators have proposed a tentative life cycle of *Morchella* (4, 20, 151). Recently, several aspects of the morel mating system and asexual stages have been elucidated (20–25, 147, 152, 153), including characterization of two mating types, the identification of heterothallism, pseudohomothallism, and unisexual reproduction, the recognition of a dominant haploid life cycle, and the discovery of two kinds of mitospores. These discoveries have advanced our understanding of the morel life cycle and have led to the reinterpretation of existing data.

However, some aspects that may be related to the life cycle still remain controversial, or understudied, such as the trophic status of morels and the role of the microbiome. Based on the isotopic evidence, Hobbie et al. (35) concluded that fire-adapted morel species are saprotrophic. Current cultivation modes also support the ideas that some successfully cultivated morel species are saprotrophic. Nevertheless, mycorrhizal-like interactions have also been found in species of *Morchella* (160, 161). So far, there is no clear final conclusion as to the trophic status of *Morchella* species. In addition, little is known of the role of the microbiome in the life cycle of morels. Some studies have indicated that 29 bacterial taxa are statistically associated with the fruiting of *M. sextelata* (41) and that the dominating fungal community can change from the early to the

cropping stage during the fruiting of *M. rufobrunnea* (42). In this review, in order to make a generalized hypothetical life cycle focusing on the mating systems, we consider the trophic status of morels to be saprotrophic, according to the results reported by Hobbie et al. (35), and we do not discuss the possible relationships among morels, plants, and the microbiome.

A hypothetical life cycle of morels is given in Fig. 5, with heterothallism, pseudohomothallism, unisexual reproduction, and asexual reproduction included. Of the three known types of ascocarps with different *MAT* distribution patterns (20), we speculate that ascocarps produced by heterothallism are likely to correspond to type II (Fig. 3A), serving as the dominant type and needing two partners to mate. Ascocarps produced by pseudohomothallism are likely to correspond to type I (Fig. 3A), resulting from a single heterokaryotic ascospore and undergoing independent sexual reproduction. We suggest that ascocarps produced by unisexual reproduction are likely to correspond to type III (Fig. 3A), independently undergoing sexual reproduction with only one mating type; although no ascospores of this type were observed (20), the possibility of rare asci with ascospores being missed cannot be entirely excluded. In asexual reproduction, mitospores (conidia and chlamydospores) are formed.

For the following hypothetical statement, we focus on heterothallism and asexual reproduction. The *Morchella* life cycle involves alternating haploid, heterokaryotic, and diploid phases and involves mitosis, plasmogamy, karyogamy, and meiosis. The life cycle commences with a mature fruiting body. Its ascus contains eight multinucleate ascospores (151), which are produced by meiosis and postmeiotic mitoses from a diploid nucleus formed by a karyogamy of two parental haploid (n , *MAT1-1* and *MAT1-2*) nuclei. These ascospores are usually haploid, with either *MAT1-1* or *MAT1-2* (20–23). Once mature, the ascospores are released and, under suitable environmental conditions, germinate, grow, and form a haploid dikaryotic mycelium. The haploid vegetative mycelia, with either *MAT1-1* or *MAT1-2*, are able to go into the asexual stages and produce conidiophores and conidia (153).

There are three pathways for conidial growth, which differ primarily in whether the conidia germinate or not. In pathway I, conidia act as spermatia, as has been suggested for other ascomycetes (55, 158, 159). In pathway II, conidia undergo vegetative growth (153) and then keep growing as reproductive propagules or act as fertilization agents in the form of hyphae. In pathway III, conidial hyphae are able to produce chlamydospores (asexual spores) (153). The chlamydospores keep dormant under adverse environmental conditions or germinate into a vegetative mycelium under suitable conditions (162). The vegetative mycelium (either *MAT1-1* or *MAT1-2*) continues growing, intertwining, and forming sclerotia rich in lipids and polysaccharides, which appear to be adapted for survival under adverse conditions (4, 30, 151, 162). Although *Morchella* sclerotia are considered to be pseudosclerotia because they lack the medulla and rind (151), the term “sclerotia” is used in many studies (4, 17, 30, 151–153, 162–164). Considering the similar function of pseudosclerotia and sclerotia, despite the differences in their development and anatomy, we use the term sclerotia here for convenience. Sclerotia can germinate again into a vegetative mycelium under certain conditions (149, 151). Volk and Leonard (151) proposed that sclerotia produced by a vegetative mycelium may develop directly into fruiting bodies. This view has been questioned by Pilz et al. (4), who deem that scenario unlikely because of the haploid nature of sclerotium produced by the vegetative mycelium.

The differentiated gametangia (ascogonia and antheridia), trichogynes, and croziers observed in other ascomycetes (56) have not yet been identified in morel species (29, 151, 165, 166). According to the trend of morphological differentiation of the ascocarp in ascomycetes (167), we speculate that the morel sexual apparatus may be reduced. In *Morchella*, the female gametes are presumably the hyphae of the haploid vegetative mycelium (either *MAT1-1* or *MAT1-2*), in which the specialized cell plays a role similar to that of the ascogonium. Male gametes with opposite mating types are presumably conidia acting as spermatia (147, 153, 156–159) or hyphae from a mycelium produced

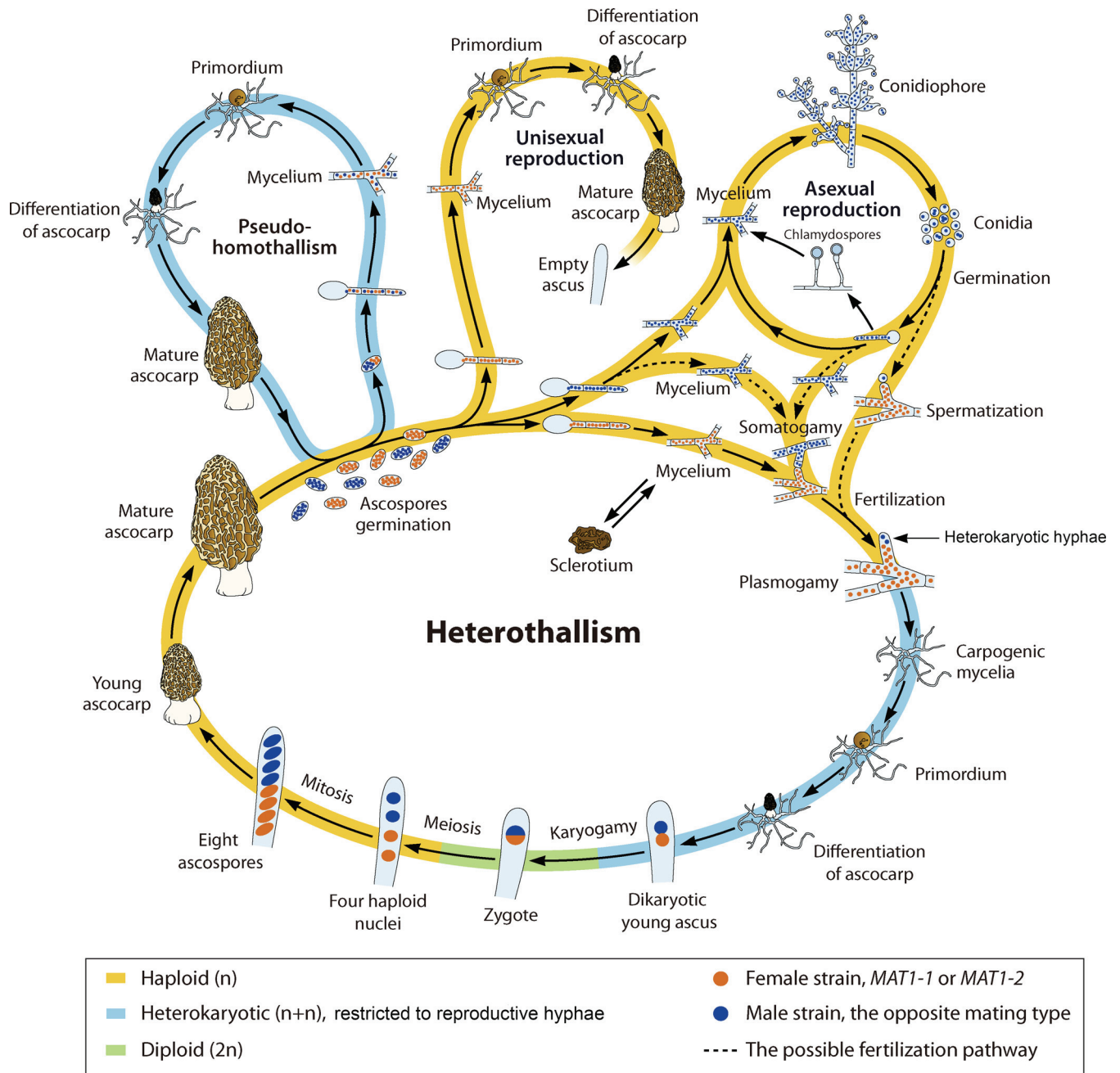


FIG 5 Generalized life cycle of *Morchella*, with heterothallism, pseudohomothallism, unisexual reproduction, and asexual reproduction included. Of the known three types of ascocarps with different *MAT* distribution patterns (20), the ascocarps produced by heterothallism are likely to correspond to type II (Fig. 3A), needing two partners with opposite mating types to mate for sexual reproduction and being the dominant strategy. The ascocarps produced by pseudohomothallism may correspond to type I (Fig. 3A), developing from a single heterokaryotic ascospore and undergoing independent sexual reproduction. The ascocarps produced by unisexual reproduction should coincide with type III (Fig. 3A), independently undergoing sexual reproduction with only one mating type, with no ascospores observed (20). In the sexual process of heterothallism, presumably the specialized cell of the female hypha receives male nuclei from one conidium via spermatization or from a specialized cell of the male hypha via somatogamy to form a heterokaryotic cell. The heterokaryotic cell probably acts as an ascogonium and produces multinucleate ascogenous hyphae. Meiosis takes place almost immediately after karyogamy in the developing asci, followed by several postmeiotic mitoses, giving rise to eight plurinucleate ascospores. The heterokaryotic phase is short in the life cycle of morels and is restricted to the reproductive hyphae, being embedded in the homokaryotic haploid vegetative mycelium. The vegetative mycelia contribute to the main structure of the ascocarp (the nonascogenous sterile tissue), and the reproductive hyphae contribute to the ascospore pools. In asexual reproduction, mitospores (conidia and chlamydospores) are formed. The dashed lines represent hypotheses of fertilization pathways that require verification.

by germinating ascospores or conidia (131, 153, 168). The latter two fertilization possibilities occur by somatogamy, also suggested in Gäumann (169) and Kirschner (170). Meiosis takes place twice almost immediately after karyogamy in the young asci produced by multinucleate ascogenous hyphae, and the nuclei divide into four haploid

nuclei, followed by one subsequent mitosis giving rise to eight nuclei (29, 151). Eight incipient ascospores with one nucleus in each spore are formed after spore delimitation (29, 151). The number of nuclei in mature ascospores and the number of subsequent mitoses occurring in incipient ascospores could change with different morel species (20, 21).

In ascomycetes, all fruiting body-forming tissues except ascospores arise from haploid, nonheterokaryotic hyphae (86). The heterokaryotic state of *Morchella* is most likely to be restricted to sexual reproductive cells. The reproductive/fertile hyphae are supported by and embedded in the haploid homokaryotic multinucleate vegetative mycelia, which contribute to the main structure of the ascocarp (the nonascogenous sterile tissue) having the same mating type (Fig. 3A and B), as indicated by the analysis of haploid stipes (representing the sterile tissue) of type II. The type II stipes, which have only one mating type (Fig. 3A) (20), have the same mating type as the haploid ridges (ectal excipulum; X.-H. Du et al., unpublished data). Development of morels does not begin from the base of the stipe followed by development of the hymenium and the ridges but rather has a fertilized hypha as the starting point, in which the haploid hyphae grow positively geotropically to form the stipe, and the fertilized and haploid vegetative hyphae grow negatively geotropically to form the hymenium and the ridges.

Some studies on morels suggest that mycelium containing both sterile and fertile hyphae may develop a sclerotium, which then could form carpogenic mycelium, or could germinate and form sterile and fertile mycelia (19, 152). To date, there is no direct evidence to support the notion that there are both sterile and fertile hyphae in a sclerotium. We have analyzed the mating-type genes in hundreds of morel sclerotia produced in cross-mating cultures of *MAT1-1* and *MAT1-2* ascospores and found that most of them harbored only one of the idiomorphs, while very few harbored both *MAT1-1* and *MAT1-2* (X.-H. Du, T. Li, and N. Xu, unpublished data). However, for the sclerotia with both *MAT1-1* and *MAT1-2*, we were not able to determine whether they formed from a mycelium containing both sterile and fertile hyphae or from a compact mass of *MAT1-1* and *MAT1-2* haploid sterile mycelia. According to Frazer (171), sclerotia are formed from vegetative hyphae, and mating-type genes do not operate in this situation. Furthermore, Horn et al. (172) reported that most sclerotia of *Aspergillus flavus* are likely to originate from single strains of one mating type. Faretra et al. (173) suggested that sclerotia of heterothallic *Botryotinia* species could be fertilized by conidia of the opposite mating type. Sclerotia formation is widely thought to be critical for morel fructification (4, 17, 19, 30, 151, 152, 162, 164, 174); however, the role and importance of sclerotia in the morel life cycle remain unclear.

Under suitable environmental conditions, carpogenic mycelium produce primordia that continue to grow and differentiate into fruiting bodies. Nevertheless, under unfavorable conditions, primordia tend to abort, partially contributing to reduced or failed production at cultivation sites in China. The specific triggers of morel fructification are currently unknown.

In summary, we have provided a generalized hypothetical life cycle of *Morchella* based on the latest new findings. We hope that this tentative model will instigate discussions on the biology of *Morchella* and lead to improvements in morel cultivation. With the rapid acceleration of morel research, morels will become readily available and inexpensive in the near future.

MORELS AS CANDIDATE MODEL ORGANISMS IN STUDYING SEXUAL DEVELOPMENT IN ASCOMYCETES

We think morel species, especially *M. importuna*, *M. sextelata*, and *M. eximia*, could be good candidates as model organisms for the future study of sexual development, mating genetics, and mating genes in ascomycetes (75, 175–177).

As an economically important research subject, morels also have worldwide geographical distribution. Importantly, they are amenable both to culture in the laboratory, with rapid ascospore germination, and to artificial cultivation, both of which

render them highly suitable for physiological and genetic studies. The current knowledge of mating systems of morel species suggests that they might be ideal models for the understanding of sexual development in *Morchella* and other ascomycetes. Furthermore, the maintenance of sexual and asexual reproduction strategies within a single organism makes morels a suitable model for understanding how sexual morphogenesis and the asexual stages are controlled and initiated in filamentous ascomycetes and for analyzing the function of mating-type genes and mating-type-regulated processes. In the near future, it might be possible to use transformation and, presumably, targeted mutation for gene replacement and for *MAT* deletion and shuffling in different genetic backgrounds, which will be useful in addressing various questions regarding the functions of *MAT* genes. Another added advantage of morels as model organisms is their production of hermaphrodite spores. These can be used to advance our understanding of the biology of sexual and asexual behavior in fungi. Finally, the sequenced genomes of morel species and the ongoing genome-sequencing projects will yield invaluable resources, particularly in facilitating the analysis of function and evolution of *MAT* genes and their roles on sexual development in *Morchella*. All these considerations support the use of morels as candidate model organisms to study various aspects of fungal biology.

CONCLUDING REMARKS

Morels are a fascinating group of fungi for studying the molecular mechanisms and evolutionary implications of sexual reproduction. To date, three kinds of sexual reproductive modes have been found in *Morchella*: heterothallism, pseudohomothallism, and unisexual reproduction (homothallism). Mixed mating systems exist in some *Morchella* species, and heterothallism is the dominant reproductive mode in this genus. Whether there are other sexual reproductive modes, and whether more species have mixed mating systems in *Morchella*, should be revealed by further studies in the future. Understanding of the mating systems of these economically important fungal species is a major issue that needs to be addressed for the better management of their life cycles, cultivation, and domestication.

APPENDIX

GLOSSARY

MAT Mating type.

MAT locus Mating-type locus.

MAT genes Mating-type genes.

idiomorph Mating-type genes are located on the *MAT* locus and share little homology, and so they are not true alleles and are termed “idiomorphs.”

heterothallism Self-incompatible; individuals have either a *MAT1-1* or *MAT1-2* idiomorph in each haploid nucleus, and mating occurs only between partners of opposite mating types to complete the sexual life cycle.

primary homothallism Self-compatible; individuals have both mating types in each haploid nucleus and are able to complete the sexual cycle without seeking a mate.

pseudohomothallism Self-compatible; individuals package nuclei of opposite mating types (each haploid nucleus having one mating type) within a single multinucleus spore and are fully able to undergo independent sexual reproduction.

mating-type switching Each haploid individual expresses only one mating type, though it carries the information of both mating types, and the “silent” copies of the alternate mating type, which reside in a transcriptionally nonactive region, are used to change the mating type identity via the transposition of information from a silent cassette to an active site.

unisexual reproduction Also termed monokaryotic, homokaryotic, or haploid fruiting, same-sex mating or single-mating-type reproduction. It occurs when a single isolate having only one mating-type gene independently undergoes sexual reproduction, with sexual processes such as meiosis and genome diploidization being involved.

silent cassette Additional copies of the two opposite mating idiomorphs in the genome, used for mating-type switching. These cassettes are transcriptionally inactive because of modified chromatin structure.

spermatium (pl. spermatia) A nonmotile male reproductive cell.

propagule Any material that functions in propagating an organism to the next stage in its life cycle.

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