

T. C. Harrington · D. L. McNew

**Self-fertility and uni-directional mating-type switching in *Ceratocystis coerulescens*, a filamentous ascomycete**

Received: 6 July 1996 / 25 March 1997

**Abstract** Individual perithecia from selfings of most *Ceratocystis* species produce both self-fertile and self-sterile progeny, apparently due to uni-directional mating-type switching. In *C. coerulescens*, male-only mutants of otherwise hermaphroditic and self-fertile strains were self-sterile and were used in crossings to demonstrate that this species has two mating-types. Only *MAT-2* strains are capable of selfing, and half of the progeny from a *MAT-2* selfing are *MAT-1*. Male-only, *MAT-2* mutants are self-sterile and cross only with *MAT-1* strains. Similarly, self-fertile strains generally cross with only *MAT-1* strains. *MAT-1* strains only cross with *MAT-2* strains and never self. It is hypothesized that the switch in mating-type during selfing is associated with a deletion of the *MAT-2* gene.

**Key words** Homothallism · Mating-type switching · Hermaphroditism · *Ceratocystis*

**Introduction**

Sexual compatibility in heterothallic ascomycetes is determined by two alleles (idiomorphs) at a single mating-type locus; two strains must be of opposite mating-type to produce ascospores (Glass and Kuldau 1992). Heterothallism encourages outbreeding, which has obvious advantages, but self-fertile species occur in many groups of ascomycetes. Various forms of homothallism are known (Beatty et al. 1994), and in yeasts bi-directional mating-type switching allows self-fertility in otherwise heterothallic species (Herskowitz 1989). It has been proposed (Mathieson 1952; Perkins 1987; Fujii and Uhm 1988) that uni-directional mating-type switching allows for selfing in

some filamentous ascomycetes. Although a switch in the expression of mating-type is seen in these fungi, it is not clear if a physical movement of mating-type genes is involved. It is also not clear if the expressed mating-types of the respective self-fertile and self-sterile progeny are homologs of the mating-type genes in other strictly heterothallic species of ascomycetes.

*Sclerotinia trifoliorum* and *Chromocrea spinulosa* show a 1:1 segregation of self-fertile and self-sterile progeny in perithecia from selfings or crosses (Mathieson 1952; Uhm and Fujii 1983a, b). In tetrad analyses of selfings or crosses, half of the ascospores in an ascus are large and give rise to self-fertile colonies, and the other ascospores are small and give rise to self-sterile colonies. Spore size appears to be a pleiotropic character associated with self-fertility. The self-fertile and self-sterile phenotypes have been referred to as differing mating-types. The self-fertile mating-type can switch to the self-sterile mating-type, but the reverse mutation does not occur. A small percentage of the single-ascospore progeny of *Botryotinia fuckeliana* may also self and give rise to progeny of two mating-types (Faretra and Pollastro 1996). A similar but more complicated form of mating-type switching may occur in *Glomerella cingulata* (Chilton and Wheeler 1949; Wheeler 1950).

Segregation for self-fertility and self-sterility is also seen in many species of *Ceratocystis sensu stricto* (exclusive of *Ophiostoma* species such as *O. ulmi*), including *Ceratocystis fimbriata*, *C. albofundus*, *C. coerulescens* and *C. coerulescens* f. *douglasii* (Andrus and Harter 1937; Olson 1949; Bakshi 1951; Davidson 1953; De Beer 1994). In *C. fimbriata*, the ratio of self-fertile to self-sterile progeny in a single perithecium varies greatly (Olson 1949; Webster and Butler 1967). Similar to the species with uni-directional mating-type switching, self-sterile progeny from a selfing of *C. fimbriata* do not produce perithecia when paired among themselves, but they can cross with self-fertile progeny (Webster 1967). *Ceratocystis eucalypti*, *C. fagacearum*, *C. radicola* and *C. paradoxa* are reported to be strictly heterothallic (no self-fertility seen), with each single-ascospore strain of one of two mating-types (Dade 1928; Hepting et al. 1952; Kile et al. 1996;

T. C. Harrington (✉) · D. L. McNew  
Department of Plant Pathology, Iowa State University,  
Ames, IA 50011, USA

Communicated by B. G. Turgeon

El-Ani et al. 1957), typical of the mating system in other filamentous ascomycetes. We (unpublished), however, have found that progeny from a self-fertile isolate of *C. paradoxa* from coconut palm in Brazil segregate for self-fertility and self-sterility.

We hypothesized that species of *Ceratocystis* expressing self-fertility have a uni-directional mating-type switching mechanism analogous to that in *S. trifoliorum* and *C. spinulosa*. Perithecia of *Ceratocystis* species show adaptations for insect dispersal (Harrington 1993), and because the ascus wall dissolves while still in the perithecium we have been unable to conduct tetrad analyses on these fungi. However, single-ascospore progeny of a single perithecium can be easily recovered from sticky ascospore masses at the tip of the perithecial neck. We (Harrington and McNew 1995) recovered both self-sterile and self-fertile progeny from perithecia of several species of *Ceratocystis*, including *C. coerulescens*, a fungus known to cause blue stain in conifer sapwood (Harrington et al. 1996). This species proved particularly amenable to mating studies. We used self-sterile, male-only mutants of otherwise hermaphroditic and self-fertile strains to demonstrate that this species has two mating-types, that only *MAT-2* strains are capable of selfing, and that half of the progeny from a *MAT-2* selfing are *MAT-1*.

## Materials and methods

**Strains.** The isolates of *C. coerulescens* (species A) studied were from blue-stained wood of *Pinus* species in England (Harrington et al. 1996). The genotypic and phenotypic characters of these iso-

lates and other laboratory derived strains are given in Table 1. Isolate C798 was self-fertile and produced an abundant aerial mycelium and protoperithecia. Isolate C490 was a self-sterile subculture from C798 that produced little aerial mycelium and few protoperithecia (designated the "flat" phenotype). Isolates C487, C488, C489 and C793 were also self-sterile but produced the typical aerial mycelium and abundant protoperithecia (designated the "aerial" phenotype). A cycloheximide-resistant mutant (C488R) was generated by exposure to UV light. A conidial suspension was filtered through a double layer of sterile cheesecloth, and 10 ml of the suspension was dispersed into 50-mm-diameter Petri plates, exposed for 40 s to UV light, and then spread onto MEA plates. Plates were incubated for 24 h in the dark and then overlaid with molten MEA with cycloheximide added at a concentration of 0.1 µg/ml. Developing colonies were recovered, and MEA with 0.1 µg/ml cycloheximide was used to evaluate resistance (growth rate as in MEA) or sensitivity (no growth).

**Crosses.** Crosses were designated by a "DM" number, and single-ascospore strains from such crosses were randomly assigned an "s" number (e.g., DM44-s27 was the 27th single-ascospore strain from DM44, a cross of C488×C490). Cultures were grown and pairings were conducted at room temperature and lighting. In most pairings, recipient and spermatizing cultures were distinguished. Recipient cultures were grown for 7–10 days on 1.5% malt extract with 2.0% agar (MEA) in 95-mm-diameter plastic Petri dishes. In some cases, an autoclaved 3-cm-long twig of *Pinus strobus* was placed in the recipient plate before the addition of molten MEA (Harrington 1992). Spermatizing cultures were grown on MYEA (2.0% malt extract, 0.2% yeast extract, and 1.5% agar), which is more suited than MEA for conidium production. The spermatizing cultures were flooded with sterile, de-ionized water. Conidia, conidiophores and mycelial fragments were loosened by lightly scraping with a spatula, and 1 or 2 ml of this suspension was added to each recipient culture. The recipient plate was swirled slightly to disperse the inoculum. Plates were incubated at room temperature and inspected periodically for perithecia and ascospore production, which was generally evident within 2 weeks, but plates were observed periodically for up to 1 month. Other pairings, particularly those involving two flat strains

**Table 1** Isolates and strains of *C. coerulescens* with their genotypic and phenotypic characters

Strain no.	Origin	Mating type	Mycelial phenotype	Ability to self	Cycloheximide resistance
C487	Field isolate	<i>MAT-1</i>	Aerial	Self-sterile	<i>CyhS</i>
C488	Field isolate	<i>MAT-1</i>	Aerial	Self-sterile	<i>CyhS</i>
C488R	Mutant of C488	<i>MAT-1</i>	Aerial	Self-sterile	<i>CyhR</i>
C489	Field isolate	<i>MAT-1</i>	Aerial	Self-sterile	<i>CyhS</i>
C490	C798 subculture	<i>MAT-2</i>	Flat	Self-sterile	<i>CyhS</i>
C793	Field isolate	<i>MAT-1</i>	Aerial	Self-sterile	<i>CyhS</i>
C795	Field isolate	<i>MAT-2</i>	Aerial	Self-fertile	<i>CyhS</i>
C798	Field isolate	<i>MAT-2</i>	Aerial	Self-fertile	<i>CyhS</i>
DM7-s1	C489×C490	<i>MAT-2</i>	Flat	Self-sterile	<i>CyhS</i>
DM7-s73	C489×C490	<i>MAT-2</i>	Flat	Self-sterile	<i>CyhS</i>
DM44-s27	C488×C490	<i>MAT-2</i>	Flat	Self-sterile	<i>CyhS</i>
DM77-s26	C488×C490 progeny backcrossed to C490	<i>MAT-1</i>	Aerial	Self-sterile	<i>CyhS</i>
DM82-s5R	C488R×C490	<i>MAT-2</i>	Flat	Self-sterile	<i>CyhR</i>
DM82-s20	C488R×C490	<i>MAT-2</i>	Aerial	Self-fertile	<i>CyhS</i>
DM82-s52R	C488R×C490	<i>MAT-2</i>	Flat	Self-sterile	<i>CyhR</i>
DM90-s28	C488×DM82-s52R	<i>MAT-2</i>	Flat	Self-sterile	<i>CyhS</i>
DM92-s39	C488×C490 progeny backcrossed to C490	<i>MAT-1</i>	Aerial	Self-sterile	<i>CyhS</i>
DM92-s48	C488×C490 progeny backcrossed to C490	<i>MAT-1</i>	Aerial	Self-sterile	<i>CyhS</i>
DM93-s62R	C488×DM82-s5R	<i>MAT-2</i>	Flat	Self-sterile	<i>CyhR</i>
DM102.P4-s9	C488R×DM82-s20	<i>MAT-2</i>	Aerial	Self-fertile	<i>CyhS</i>
DM102.P4-s13	C488R×DM82-s20	<i>MAT-2</i>	Aerial	Self-fertile	<i>CyhS</i>
DM183-s51	C488×DM93-s62R	<i>MAT-2</i>	Flat	Self-sterile	<i>CyhS</i>

(strains with less aerial mycelium generally mated poorly), were conducted without regard to which strain served as the recipient or spermatizing partner. In these cases, spermatizing suspensions of two strains were mixed in equal volumes, and about 1 ml of this suspension was added to a fresh plate of MEA or twig medium and swirled. Self-fertility of single-ascospore strains was evaluated by incubating on MEA at room temperature and lighting. Perithecia production was noted at 3 weeks. Aerial and flat phenotypes of strains were also distinguished on MEA.

**Isolation of ascospores.** Ascospores of *Ceratocystis* species do not disperse well in water (Whitney and Blauel 1972) but will disperse in oil. Single-ascospore strains were obtained by collecting a mass of exuded ascospores from the tip of a perithecial neck and dispersing in about 40  $\mu$ l of Isopar M (a light sulfur oil, Exxon Corporation) in a deep-well glass slide. Generally, about 5  $\mu$ l of this suspension was diluted in about 40  $\mu$ l of Isopar M, and a small amount of this spore suspension was picked up with a wire loop and streaked over a 1.5% malt-agar plate. This plate was incubated at room temperature for 48 h, after which individual germlings were excised and transferred to 1.5% malt-agar. Dilute spore suspensions proved to be important in recovering representative populations of germlings, without bias for speed of germination or growth rate.

## Results

### Self-fertile and self-sterile progeny from selfings

Among seven wild isolates of *C. coerulescens* examined, only C795 and C798 were self-fertile (see Table 1). Among the single-ascospore progeny recovered from a single perithecium of C798, 75 were self-fertile and 72 were self-sterile, closely fitting a 1:1 segregation ratio. All progeny of C798 retained the aerial mycelial phenotype of the parent isolate.

Self-fertile strains generated from crosses of self-sterile strains also produced self-fertile and self-sterile progeny. The self-sterile isolate C490, a subculture of C798 that had the flat mycelial phenotype, crossed with self-sterile isolates with the aerial phenotype, and some of the aerial phenotype progeny from such crosses were self-fertile. A total of 538 single-ascospore progeny were recovered from selfings of self-fertile strains generated from ten crosses between C488 $\times$ C490 or C489 $\times$ C490. All of the progeny from these selfings were of the aerial phenotype, like the parent strain, and 383 were self-fertile. The ratio of self-fertile to self-sterile progeny was significantly greater than that expected for a 1:1 segregation. We typically found this deviation from 1:1 only in progeny recovered from dense lawns of ascospores, apparently because of bias in sampling only the earliest-germinating progeny. When more dilute ascospore suspensions were used and germlings harvested later, 1:1 segregation for self-fertility and self-sterility was seen in selfings of *C. coerulescens*, which would be expected if selfing is due to a uni-directional mating-type switch.

### Two mating-types among self-sterile strains

Four self-sterile isolates of *C. coerulescens* showed evidence of two mating-types when paired among themselves

**Table 2** Ascospore production in crosses between self-sterile isolates of *C. coerulescens*

Spermatizing isolates	Recipient isolates			
	C490 <i>MAT-2</i>	C487 <i>MAT-1</i>	C488 <i>MAT-1</i>	C489 <i>MAT-1</i>
C490	0/3 <sup>a</sup>	3/3	5/5	5/5
C487	3/3	0/3	0/3	0/3
C488	1/3	0/3	0/3	0/3
C489	0/3	0/3	0/3	0/3

<sup>a</sup> Ratio of crosses with ascospore masses produced/attempted crosses

(Table 2). Isolate C490 and the self-fertile C798, from which C490 was subcultured, were arbitrarily designated *MAT-2*, while C487, C488 and C489 were designated *MAT-1*. When used as recipients, the *MAT-1* isolates consistently (13 positive out of 13 attempts) produced ascospores when spermatized by C490. However, C490 as a recipient produced fewer protoperithecia and perithecia than the other strains, and ascospores were produced in only 4 of 9 cases where it was spermatized by *MAT-1* isolates.

Because of its poor function as a female, we refer to C490 as a male strain, while C798, C487, C488 and C489 were designated as hermaphrodites. In contrast to the other isolates, C490 had a "flat" mycelial morphology. This phenotype had few protoperithecia, and these often took up to 3 weeks to develop, while the hermaphroditic isolates ("aerial" phenotype) produced protoperithecia in less than 1 week. Also, the flat phenotype produced fewer conidiophores and less aerial mycelium, especially at the margins of the plate, whereas the aerial phenotype produced many conidiophores and an aerial mycelium spread uniformly across the plate.

Two mating-types were identified among the self-sterile progeny recovered from a single perithecium of the cross C489 $\times$ C490 (designated DM7). Twelve of the DM7 progeny had the aerial phenotype and were self-fertile, six had the aerial phenotype but were self-sterile, and 52 had the flat phenotype. All of the flat-phenotype progeny were self-sterile. The six aerial, self-sterile progeny and 12 randomly selected flat progeny were paired in all combinations, with each strain used both as a recipient and as a spermatizing strain (Table 3). Six of the flat progeny were compatible with the self-sterile, aerial progeny and with *MAT-1* C489, and these progeny were designated *MAT-2*. The other six strains with the flat phenotype mated with only the flat, *MAT-2* progeny, and were thus identified as *MAT-1*. All of the self-sterile, aerial progeny were *MAT-1*.

Both mating-types were identified among progeny recovered from crosses between flat strains of opposite mating-type. Two *MAT-1*, flat-phenotype strains from the cross C488 $\times$ C490 (DM44) were crossed with C490 or DM44-s27, both of which were *MAT-2*, flat. Each of 115 progeny recovered from three crosses were of the flat phenotype, and none was self-fertile. Based on pairings with *MAT-1* (C488) and *MAT-2* (DM7s-1) tester strains, 56 of

**Table 3** Perithecium (P) and ascospore (A) production in crosses between aerial and flat, self-sterile progeny from the cross C489 (*MAT-1*, aerial)×C490 (*MAT-2*, flat) of *C. coerulescens*

Spermatizing strains	Recipient strains																	
	<i>MAT-1</i> , aerial						<i>MAT-1</i> , flat						<i>MAT-2</i> , flat					
	13	16	21	28	60	69	2	7	15	34	35	44	1	6	22	39	57	73
13	– <sup>a</sup>	–	–	–	–	–	–	–	–	–	–	–	A	A	A	A	A	A
16	–	–	–	–	–	–	–	–	–	–	–	–	A	A	A	A	A	A
21	–	–	–	–	–	–	–	–	–	–	–	–	A	A	A	A	A	A
28	–	–	–	–	–	–	–	–	–	–	–	–	A	A	A	A	A	A
60	–	–	–	–	–	–	–	–	–	–	–	–	A	A	A	A	A	A
69	–	–	–	–	–	–	–	–	–	–	–	–	A	A	A	A	A	A
2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	A	–
7	–	–	–	–	–	–	–	–	–	–	–	–	A	A	–	A	A	P
15	–	–	–	–	–	–	–	–	–	–	–	–	A	A	A	A	A	–
34	–	–	–	–	–	–	–	–	–	–	–	–	A	A	A	A	A	–
35	–	–	–	–	–	–	–	–	–	–	–	–	A	A	P	A	A	P
44	–	–	–	–	–	–	–	–	–	–	–	–	P	–	A	–	–	–
1	A	A	A	A	A	A	A	A	A	A	A	A	–	–	–	–	–	–
6	A	A	A	A	A	A	A	A	A	A	A	A	–	–	–	–	–	–
22	A	A	A	A	A	A	A	P	A	A	A	–	–	–	–	–	–	–
39	A	A	A	A	A	A	A	A	A	A	A	P	–	–	–	–	–	–
57	A	A	A	A	A	A	A	A	A	P	A	–	–	–	–	–	–	–
73	A	A	A	A	A	A	A	A	A	A	A	A	–	–	–	–	–	–

<sup>a</sup> No perithecia (–), sterile perithecia (P), or perithecia with ascospores (A)

the progeny were *MAT-1* and 59 were *MAT-2*, thus showing a 1:1 segregation ( $P = 0.05$ ) for mating-type.

#### Flat phenotype and infertility

The aerial phenotype was strongly associated with reproductive ability or fertility in crosses, and appeared to be essential for selfings to occur. Relatively few perithecia, usually less than ten per plate, developed in the positive pairings between flat-phenotype isolates of opposite mating-type, and in some cases mature (full-sized) perithecia developed but no ascospore masses were exuded. The flat *MAT-2* strains were particularly poor as recipient strains. Strain DM7-s73 served only as a male when crossed with flat *MAT-1* strains; the reciprocal crosses did not result in perithecia (Table 3). In contrast, more than 100 perithecia with ascospores formed in each of the 36 plates where the aerial progeny served as the recipient strain in compatible pairings (Table 3).

Only strains with the aerial phenotype were self-fertile, and we were unable to generate self-fertile progeny from crosses of flat strains of opposite mating-type. Each of 1360 progeny from 36 crosses between *MAT-1*, flat strains (DM7 or DM44 progeny) and *MAT-2*, flat strains (C490) was of the flat phenotype. Only 7 of these 1360 progeny produced perithecia through selfing, and only 1–3 perithecia formed in the plates of the seven exceptional progeny. Ascospore masses were seen on the top of six of these perithecia, and a total of 250 single-ascospore progeny were recovered from four of them. None of these 250 progeny

was of the aerial phenotype, and only one produced perithecia. This strain produced two perithecia, one with no ascospores and another with an ascospore mass and, when this mass was transferred to another plate, no perithecia were produced on the mycelium that developed. Thus, progeny from crosses of flat strains were of the flat phenotype and essentially self-sterile. In the rare cases where perithecia did form without pairing, these perithecia were very few in number and often produced few viable ascospores. However, these flat isolates did form normal perithecia with ascospores, however, when paired with strains of the opposite mating-type.

#### *MAT-2*, flat×*MAT-1*, aerial pairings

Segregation for mycelial phenotype and the ability to self was examined in pairings between *MAT-2*, flat strains (C490, as well as DM93-s62R and DM183-s51, from crosses similar to DM7) with C488 and other *MAT-1*, aerial strains. Single-ascospore progeny were recovered from seven perithecia. Half of the 661 progeny examined had the aerial phenotype and half had the flat phenotype (Table 4), showing that this character segregates as a single gene. Of the aerial phenotypes, half were self-fertile and half were self-sterile. All of the flat progeny were self-sterile. The 1:1:2 segregation ratio (Table 4) is that expected for two factors (the *MAT-2* mating-type and the aerial phenotype) required for self-fertility, and these factors are unlinked.

**Table 4** Segregation of self-fertility and mycelial phenotype in single-ascospore progeny from individual perithecia from crosses of self-sterile strains (*MAT-1*, aerial and *MAT-2*, flat) of *C. coerulescens*.

Parents		Number of Progeny <sup>a</sup>		
Aerial <i>MAT-1</i>	Flat <i>MAT-2</i>	Aerial self-fertile	Aerial self-sterile	Flat self-sterile
DM92-s48	C490	21	25	53
DM92-s48	C490	28	25	48
DM92-s39	C490	26	18	56
DM77-s26	C490	22	21	34
C793	C490	24	29	44
C488	DM93-s62R	17	23	52
C488	DM183-s51	28	24	43
Total		166	165	330

<sup>a</sup> Aerial:flat phenotypes and aerial, self-fertile:aerial, self-sterile segregated in 1:1 ratios among progeny of each cross (chi-square,  $P = 0.05$ )

Segregation for mycelial phenotype and mating-type was examined in 96 single-ascospore progeny recovered from a cross between C490 (flat, *MAT-2*) and DM92-s48 (aerial, *MAT-1*). Based on mycelial morphology on MEA and pairings of the flat phenotypes with tester strains of each mating-type, 28 of those 96 progeny were aerial, self-fertile (presumably *MAT-2*); 25 were aerial, self-sterile (presumably *MAT-1*); 21 were flat, *MAT-1*; and 22 were flat, *MAT-2*. This segregation closely ( $P = 0.05$ ) fits the 1:1:1:1 ratio expected for independent segregation of mycelial phenotype and mating-type.

Two crosses similar to the above were conducted with either the *MAT-1*, aerial or the *MAT-2*, flat parent carrying cycloheximide resistance. Analyses of the progeny from each of the two crosses showed independent assortment of mating-type, mycelial phenotype, and cycloheximide resistance (Table 5).

In the above crosses, independent assortment of markers from each parent was seen in the progeny, but in one case we observed an induction of selfing in a flat, *MAT-2* strain. A cycloheximide-resistant, *MAT-1*, aerial strain (C488R) was crossed with a *MAT-2*, flat strain that was

sensitive to cycloheximide (DM90s-28), and all of the progeny were self-sterile and showed sensitivity to cycloheximide. Ninety nine of the one hundred progeny showed the flat phenotype of the *MAT-2* parent, and one of the progeny showed an ambiguous mycelial phenotype. Of the 56 progeny for which mating-type was determined, 26 were *MAT-1* and 30 were *MAT-2*, which fits ( $P = 0.05$ ) the expected 1:1 segregation for mating-type. The fact that the progeny differed only in mating-type suggests that the aerial, *MAT-1* parent induced selfing (mating-type switching) of the flat, *MAT-2* parent.

#### Mating type of self-fertile strains

We compared the ability of self-fertile strains to function as *MAT-2* or *MAT-1* in crosses. The *MAT-2* function of 13 self-fertile strains (self-fertile progeny from the cross DM180, C488R × C490) was tested by spermatizing a *MAT-1*, aerial strain with resistance to cycloheximide (C488R) on cycloheximide medium. Because only the *MAT-1* recipient strain had resistance to cycloheximide, the spermatizing strain could not self on the cycloheximide medium. Crossing did occur in each of the 13 pairings because both cycloheximide-resistant and -sensitive progeny were recovered from each of the perithecia sampled. All 287 of the recovered progeny from 13 perithecia (one perithecium from each cross) had the aerial phenotype of the parents, 68 were self-fertile and cycloheximide-resistant, 81 were self-fertile and cycloheximide-sensitive, 73 were self-sterile and cycloheximide-resistant, and 65 were self-sterile and cycloheximide-sensitive. This 1:1:1:1 segregation ratio ( $P = 0.05$ ) indicates that self-fertility (mating-type) and cycloheximide resistance segregate independently, and that the self-fertile strain acted as a *MAT-2* in each of the crosses.

In order to determine if self-fertile strains could also function as *MAT-1* in a cross, 12 of the same self-fertile strains (from DM180) were used to spermatize a flat, *MAT-2*, cycloheximide-resistant strain (DM93s-62R) growing on cycloheximide medium. As in the preceding crosses, the recipient strain had cycloheximide resistance while the spermatizing strains were sensitive, so the self-fertile strains could not self on the cycloheximide medium.

**Table 5** Segregation of self-fertility, mycelial phenotype, mating-type and cycloheximide resistance in single-ascospore strains from crosses of self-sterile isolates of *C. coerulescens* of opposite mating-type with differing mycelial phenotype and cycloheximide resistance

Parents		Aerial phenotype <sup>a</sup>				Flat phenotype			
		Number self-fertile		Number <i>MAT-1</i>		Number <i>MAT-1</i>		Number <i>MAT-2</i>	
<i>MAT-1</i> , aerial	<i>MAT-2</i> , flat	<i>CyhR</i>	<i>CyhS</i>	<i>CyhR</i>	<i>CyhS</i>	<i>CyhR</i>	<i>CyhS</i>	<i>CyhR</i>	<i>CyhS</i>
C488	DM93-s62R	16	15	10	14	8	15	9	13
C488R	C490	9	14	8	18	12	10	11	15

<sup>a</sup> In each cross, segregation for the three characters (cycloheximide resistance, mycelial phenotype, and mating-type) occurred independently and did not differ significantly ( $P = 0.05$ ) from the expected 1:1:1:1:1:1:1:1:1:1

None of these pairings resulted in perithecia, thus indicating that the self-fertile strains could not function as *MAT-1*.

Further attempts to cross self-fertile strains and flat, (self-sterile) *MAT-2* strains utilized the above flat, *MAT-2*, cycloheximide-resistant strain (DM93s-62R) growing on cycloheximide medium and one of two self-fertile, cycloheximide-sensitive strains (DM102.P4s-9 or DM102.P4s-13). In one such pairing, a few perithecia were produced, but the ascospore masses that developed on the few perithecia were small, and a low germination rate of the ascospores was seen. Analysis of 39 recovered progeny showed that 17 had the aerial phenotype, and 11 of these were self-fertile. Thirteen of the twenty two flat progeny were *MAT-1* and nine were *MAT-2*. Of 39 progeny recovered, 19 were resistant to cycloheximide and 20 were sensitive, and resistance was found in each of the classes noted above. Thus, a self-fertile strain was able to spermatize a *MAT-2*, flat strain in 1 out of the 14 attempted crosses, but in this exceptional case few perithecia and viable ascospores were produced. Analyses of the progeny showed independent assortment of mating-type, mycelial phenotype, and cycloheximide resistance, confirming that a cross had occurred.

Lastly, 15 self-fertile and 15 self-sterile strains sensitive to cycloheximide were recovered from a selfing of isolate C795, and these were used as spermatizing strains against cycloheximide-resistant strains on cycloheximide medium. Again, the 15 self-sterile strains functioned only as *MAT-1* in crosses, and the 15 self-fertile strains functioned well as *MAT-2* parents but not as *MAT-1* parents in crosses. When an aerial, *MAT-1* strain (C488R) was used as the recipient, no perithecia were produced when the self-sterile strains were used as spermatizing strains, but perithecia with ascospores were produced in 45 out of 45 of the attempted crosses with the self-fertile strains. When a flat, *MAT-2* strain (DM93-62R) was used as the recipient, 28 out of 45 crosses with the self-sterile strains resulted in perithecia with ascospores, but only 1 of 45 crosses with the self-fertile strains resulted in a perithecium with an ascospore mass. Fifty four progeny from this perithecium were recovered, and analyses showed that mating-type, mycelial phenotype, and cycloheximide resistance each segregated independently and in a 1:1 ratio. Thus, in this one case, a self-fertile strain functioned as a *MAT-1* in a cross, but self-fertile strains generally function only as *MAT-2* in crosses.

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## Discussion

Two mating-types can be identified in the heterothallic species *C. coerulescens*, but one of the mating-types, arbitrarily designated *MAT-2*, is typically self-fertile and gives rise to both *MAT-2* (self-fertile) and *MAT-1* (self-sterile) progeny. The switch in expression of mating-type in self-fertile strains is uni-directional, i.e., *MAT-2* strains can switch to *MAT-1* and self, but *MAT-1* progeny cannot switch to *MAT-2* and thus remain self-sterile. This provides the

strongest evidence to-date that unidirectional mating-type switching occurs in ascomycetes.

The change in expression of mating-type does not appear to occur in the vegetative phase of the fungus. We have observed that single-conidium progeny of *MAT-2* and *MAT-1* strains of *C. coerulescens* always retain the mating-type of the parent strain (unpublished); Webster and Butler (1967) observed the same phenomenon in *C. fimbriata*. Generally, self-fertile strains did not function as *MAT-1* when used as spermatizing strains, and only *MAT-2* strains capable of protoperithecium production (the aerial phenotype) were able to self. Thus, the switch in mating-type may occur in the protoperithecium, perhaps before dikaryon formation.

We assume that the two mating-types in *Ceratocystis* are idiomorphs at a single locus, which is typical for other heterothallic ascomycetes (Glass and Kuldau 1992). In other work, we (Harrington and McNew 1995) have found that *C. coerulescens* strains are partially interfertile with strains of other "self-fertile" species, a strictly heterothallic species (*C. eucalypti*), and putatively asexual species (e.g., *Chalara neocalidoniae*) in the *C. coerulescens* complex (Harrington 1996), but only if the paired strains are of opposite mating-type. Because the two mating-types in species of *Ceratocystis* with self-fertility are functionally compatible with the two mating-types in other members of this genus, we believe the two mating-types are homologous; hence the designation of *MAT-1* and *MAT-2* for the mating-types of the self-fertile species.

Using the polymerase chain reaction and degenerative primers designed for the High Mobility Group (HMG) box of the *mt a-1* sequence in *Neurospora crassa* (Arie et al. 1997), we (Strydom et al., unpublished data) amplified a product of approximately 300 bp from DNA of *MAT-2*, but not *MAT-1*, strains of *C. eucalypti*, *Chalara australis* and *Ch. neocalidoniae*. Internal primers specific to the *C. eucalypti* *MAT-2* sequence were designed, and these also specifically amplified a portion of the *MAT-2* idiomorph from DNA of *C. eucalypti* and the closely related *Chalara* species. Unfortunately, the degenerative primers and the primers designed from the *C. eucalypti* *MAT-2* sequence did not amplify a single 300-bp product from DNA of the *C. coerulescens* *MAT-2* mating-type. However, the *C. eucalypti* primers amplified the expected fragment from the DNA of self-fertile isolates and other *MAT-2* isolates of *C. virescens*, a species closely related to *C. eucalypti* that also undergoes mating-type switching (Harrington and McNew 1995). No such fragment was produced with the DNA from a *MAT-1*, self-sterile isolate of *C. virescens*. We also recovered self-fertile and self-sterile progeny from the selfing of a self-fertile isolate of *C. virescens*, and the *C. eucalypti* primers amplified the expected fragment from DNA of the ten self-fertile progeny, but no fragment was amplified from DNA of the ten self-sterile progeny. Thus, we have demonstrated with these primers that at least a portion of the *MAT-2* idiomorph is deleted in a selfing event.

True homothallic species of other genera of filamentous ascomycetes generally carry the idiomorphs of both

mating-types (Beatty et al. 1994; Turgeon et al. 1995), and we hypothesize that *MAT-2* strains of ascomycetes with unidirectional mating-type switching carry both the *MAT-2* and *MAT-1* idiomorphs, while *MAT-1* strains carry only the *MAT-1* idiomorph. The preliminary findings using *MAT-2*-specific primers, along with the results from numerous pairings, support this hypothesis. Selfings of *MAT-2* strains produce progeny of both mating-types, and self-fertile strains can cross with both *MAT-1* and *MAT-2* strains, although the latter cross is apparently rare. In contrast, *MAT-1* strains do not function as *MAT-2* in crosses and do not self, so the change in expression of mating-type appears irreversible, perhaps due to a simple deletion of the *MAT-2* idiomorph.

The presence of *MAT-2* and *MAT-1* in self-fertile strains is not likely to be due to diploid nuclei or dikaryons in ascospores. Analyses of 98 isolates of *C. coerulescens* and related species for ten isozyme loci showed no evidence of heterozygosity (Harrington et al. 1996), and no heterozygosity was seen in analyses of *C. coerulescens* progeny from crosses of parents differing in isozyme electromorphs (unpublished data). Also, microscopic examination has shown that ascospores of this species are uninucleate (unpublished data). Thus, the species is apparently haploid, and single-ascospore progeny are homokaryotic. Aneuploidy is a possibility, however, and deletion of the chromosome, or part of the chromosome, with the *MAT-2* idiomorph may be responsible for the change in mating-type expression.

It is noteworthy that in the other filamentous ascomycetes with unidirectional mating-type switching, self-fertile strains arise from ascospores that are larger than the ascospores that give rise to self-sterile strains (Mathieson 1952; Ulm and Fujii 1983a), perhaps due to higher DNA content in the nuclei of the self-fertile strains. We have not seen evidence for two different sizes of ascospores in *Ceratocystis*, but we (Harrington and McNew 1995) have seen differences in growth rate between the two mating-types, with *MAT-2* strains growing significantly faster than *MAT-1* strains in all the tested species of *Ceratocystis* with mating-type switching. Like the smaller ascospore size in the other genera, the slower growth rate seen in self-sterile progeny of *Ceratocystis* species is a pleiotropic character associated with mating-type and may be due to a loss of genetic material or other chromosome rearrangement.

It is not clear if the *MAT-1* idiomorph is present as a silent copy in self-fertile strains or if a translocation of this copy is necessary for selfing to take place, in a manner similar to that in yeasts with mating-type switching (Herskowitz 1989). If physical movement of DNA does take place, it is apparently irreversible, unlike that in yeasts. In our proposed model, idiomorphs of both mating-types may be in functional locations in self-fertile strains, and the apparent shift in expression of mating-type to *MAT-1* is merely a loss in function of the gene(s) at the *MAT-2* idiomorph, perhaps by deletion. Such a deletion might be expected to have pleiotropic effects, such as the slower growth rate of *MAT-1* strains when compared to *MAT-2* strains (Harrington and McNew 1995). Strains of heterothallic filamentous

ascomycetes that have been transformed with a gene of opposite mating-type are capable of selfing, i.e., producing pseudothecia or perithecia but they are sterile (no asci or ascospores) (Picard et al. 1991; Turgeon et al. 1993). The transgene confers the ability to form pseudothecia but not asci and ascospores in crosses, if the resident *MAT* gene in the transgenic strain is suppressed (Turgeon et al. 1993; Wirsal et al. 1996). Self-fertile strains of *C. coerulescens* can self but generally only function as *MAT-2* in crosses, perhaps because they remain functionally *MAT-2* until the *MAT-2* gene is deleted, sometime after protoperithecium production. The precise stage of the switch in mating-type is not known, but it may be before the formation of the dikaryon. The expression of the *MAT-1* idiomorph would then allow for complementation with an "unswitched" nucleus, so that the sexual cycle proceeds, but half of the progeny only express the *MAT-1* mating-type and cannot revert to *MAT-2*.

Our findings with *C. coerulescens* are consistent with the reports of Olson (1949), Webster (1967), and Webster and Butler (1967) for *C. fimbriata*, in which self-sterile to self-fertile progeny are recovered from a single-ascospore mass, although the self-fertile progeny typically outnumber the self-sterile progeny in that species. In *C. coerulescens*, *C. fimbriata* and other *Ceratocystis* species, we have seen some segregation ratios that are slightly skewed, generally in favor of self-fertile progeny, and the mechanism for that skewness is not clear. In *C. coerulescens*, we often saw skewed segregation for mating-type and other characters when we recovered germlings from dense lawns of ascospores, presumably because only hyphae of the earliest germinating ascospores could be recovered before intermingling. In *C. fimbriata*, germination percentages of ascospores are generally low, and self-sterile germlings may have a lower viability or the ascospores may germinate slower than self-fertile ascospores, resulting in recovery of fewer self-sterile progeny. We (Harrington and McNew 1995) have noted that colonies of *MAT-2* strains of *C. fimbriata* have a faster linear growth rate than colonies of *MAT-1* strains. Alternatively, in the developing perithecia from selfings or crosses, the ascogenous system may be a mixture of dikaryotic hyphae, some with *MAT-1* and *MAT-2* nuclei paired and others with only *MAT-2* nuclei. Regardless of this, normal segregation ratios for mating-type and other markers were generally seen in *C. coerulescens* when sparse lawns of ascospores were used to recover individual germlings, which we feel is better suited to unbiased recovery of fast- and slow-germinating progeny.

Although isolates of *C. coerulescens* and other *Ceratocystis* species are typically hermaphroditic, we have been able to identify male mutants in many of these species. It is not clear if such male mutants are common in nature or are an artifact of laboratory culture. Male mutants were earlier reported in *C. fimbriata* and *C. fagacearum* cultures (Barnett 1953; Webster and Butler 1967), and loss of "femaleness" is apparently common in ascomycetes (Hansen 1938; Hansen and Snyder 1943; Notteghem and Silué 1992; Linders and Van der Aa 1995). In *Ceratocystis* these

mutants generally produce few protoperithecia, and in *C. coerulescens* fewer conidiophores and less aerial mycelium are seen in the male (flat) mutants than in wild-type (aerial) isolates. We rarely saw *MAT-2*, flat isolates self, and in the one time this was studied in detail it was apparently due to a mating-type switching event that was induced by pairing with a *MAT-1* strain. Regardless of mating-type, the flat phenotype is usually associated with low fertility, particularly when serving as a female. These mutations must impede the expression of many genes, but the factor is not linked to the mating-type locus.

Whatever the mechanism for selfing, it is apparently common in the genus *Ceratocystis* and appears similar to that noted in a few other filamentous ascomycetes (Mathieson 1952; Uhm and Fujii 1983a, b; Perkins 1987; Fujii and Uhm 1988; Rehnstrom and Free 1993). Further study is needed to determine the molecular mechanism of mating-type switching in *Ceratocystis* and other filamentous ascomycetes. With cloned fragments of the *MAT-2* idiomorph we will be able to more precisely follow the events leading to changes in mating-type.

**Acknowledgements** The helpful comments of Roger Wise and Charlotte Bronson are greatly appreciated. Gillian Turgeon generously provided the sequences of the degenerative primers used to amplify the *MAT-2* HMG box in *C. eucalypti*, and Corli Strydom assisted with the PCR work. Journal Paper No. J-17002 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 3226, supported by Hatch Act and State of Iowa Funds.

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