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# Localization of the Mating Type Gene in *Agaricus bisporus*

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**The cultivated mushroom *Agaricus bisporus* is secondarily homothallic. Most basidia produce two basidiospores, each of which receives two of the four postmeiotic nuclei. Usually, the two packaged nuclei carry compatible mating types. Previous studies suggested that there may be only a single mating type locus in *A. bisporus*. In this study, we determined whether the mating type segregated as a single Mendelian determinant in a cross marked with 64 segregating molecular markers. To score mating types, each of the 52 homokaryotic offspring from this cross was paired with each of the two progenitor homokaryons. Compatible matings were identified by the formation of genetically stable heterokaryons which were verified by assay of restriction fragment length polymorphisms (RFLPs). Data for screening mycelial interactions on petri plates as well as fruit body formation were compared with the RFLP results. Mating types of 43 of the 52 homokaryotic offspring were determined on the basis of RFLP analysis. Our results indicate (i) there is a segregating mating type gene in *A. bisporus*, (ii) this mating type gene is on the largest linkage group (chromosome I), (iii) mycelial interactions on petri plates were associated with heterokaryon formation under selected conditions, (iv) fruit body formation was dependent upon the mating type gene, and (v) compatible mating types may not always be sufficient for fruiting.**

Incompatibility systems are widespread in the higher fungi. Known systems in the basidiomycetes are controlled by one or two unlinked genetic factors (31). In the genus *Agaricus*, putative homothallic and heterothallic sexual systems have been reported (7, 29). One four-spored species, *A. silvicola* (7), was determined to be homothallic by the absence of successful matings among basidiospores from the same fruit body and by the ability of single-spore isolates to fruit. The common field mushroom, *A. campestris*, may include both homothallic (17) and heterothallic forms (26). Unifactorial heterothallism was reported in *A. bitorquis* (28), *A. macrosporus*, *A. nivescens* (7), and *A. vaporarius* (2). The cultivated button mushroom, *A. bisporus* (= *A. brunnescens*) (13, 23, 24), was reported to be secondarily homothallic with a unifactorial sexual incompatibility system (6, 27, 30).

In most strains of *A. bisporus*, the majority of basidia produce only two basidiospores, although low percentages of basidia bearing from one to seven spores have been reported (34). The average number of spores per basidium varies somewhat among different strains and may also vary slightly at different developmental stages of a given strain (8, 15). Spores from bisporic basidia usually contain two of the four postmeiotic nuclei, and each pair of nuclei are usually nonsisters with respect to meiosis II (35). Upon germination, these basidiospores form heterokaryotic mycelia that are characteristically capable of fruiting (9). Other, uncommon basidiospores, usually from rare three- or four-spored basidia, receive only one nucleus and form a homokaryon upon germination. These homokaryons can mate with another homokaryon carrying a compatible mating type to form a stable heterokaryon. The syndrome of producing heterothallic and secondarily homothallic spores has been described as amphithallism (19).

Our goals in this study were (i) to confirm the existence of a single segregating mating type locus in 52 homokaryotic offspring from a cross genetically marked by 64 segregating allozyme restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA markers (16) and (ii) to place the mating type locus of *A. bisporus* on the genetic map inferred previously from the segregation data (16). We also investigated (i) the association between visible mycelial interaction and heterokaryosis in these particular backcrosses and (ii) the relationship between the mating type gene and fruiting.

## MATERIALS AND METHODS

**Cultures and growing conditions.** Heterokaryon Ag93b is derived from a laboratory cross between Ag1-1 (ATCC 24662), a mutant carrying an unknown auxotrophic requirement, and Ag89-65, a homokaryon recovered by protoplast regeneration from strain Ag89, collected in an urban field site by D. Malloch (5). A spore print from Ag93b was the source of the 52 homokaryotic offspring. These 52 homokaryons were identified by loss of heteroallelism at all loci marked by allozymes and RFLPs (14). A genetic linkage map with 64 markers has been constructed from these 52 homokaryons (16). The 52 homokaryotic offspring and two progenitor homokaryons were stored in liquid nitrogen and cultured on complete yeast medium (CYM [30]) before tests for mating and fruiting were conducted.

**Mating tests.** In a previous study, media with diluted CYM amended with extracts of compost or fruiting bodies allowed the expression of mycelial interactions and heterokaryosis in more pairings of homokaryons than did full-strength, unamended CYM (unpublished data). All pairings in this study were made on diluted CYM plus spawn extract (DCS) medium containing 100 ml of liquid CYM, 200 ml of a water extract of phase II compost freshly inoculated with spawn (400 g of compost with spawn in 1 liter of distilled water,

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TABLE 1. Genotypes of the two homokaryotic progenitors

| Probe | Chromosomal location[a] | Size (kb) of *Eco*RI fragment: | |
|---|---|---|---|
| | | Ag1-1 | Ag89-65 |
| Ag4n6 | II | 5.8 | 6.9 |
| Ag4n14 | XII | 1.8 | 2.1 |
| Ag33n5 | III | 6.7 | 6.0 |
| Ag33n10 | VI | 6.3 and 1.4[b] | 6.7 and 1.4[b] |
| Ag33n25 | V | 2.4[c] | 1.4[c] |

[a] As described by Kerrigan et al. (16).
[b] The 6.3- and 6.7-kb fragments represent alleles of one locus. The 1.4- and 1.7-kb fragments represent alleles at another locus.
[c] Additional fragments hybridize to this probe. Only the fragments listed were scored in tests of heterokaryosis.

autoclaved for 40 min, with solids discarded), 700 ml of distilled water, and 15 g of agar. To test for mycelial interactions, two 2-mm$^2$ plugs of agar and mycelium were placed 2 cm apart on petri plates containing DCS medium. Cultures were grown at room temperature (21 ± 1°C) for 4 to 5 weeks, and mycelial interactions were recorded as positive, negative, or ambiguous. Each pairing was repeated at least once.

**RFLP analysis.** To avoid sampling mixed homokaryons rather than a stable heterokaryon, three pieces of agar from the junction zone of each pairing were transferred onto solid CYM. Each of the three colonies was subcultured from the margin twice in succession at 3-week intervals. Final subcultures were then used to inoculate liquid CYM in petri plates. After 3 to 4 weeks of incubation, mycelium was harvested and freeze-dried. DNA extraction was performed according to the method of Zolan and Pukkila (38). Total genomic DNA was digested with *Eco*RI, subjected to electrophoresis in 0.8% agarose gels in TAE buffer (25), and transferred onto a nylon membrane (GeneScreen Plus; DuPont) by capillary blotting. Phenotypes for RFLP markers were determined by hybridization of [$\alpha$-$^{32}$P]dCTP-labelled recombinant plasmid DNAs to membrane-bound genomic DNA fragments followed by autoradiography (4, 25). Probes contained inserts of *Eco*RI fragments of nuclear DNA from the heterokaryons *A. bisporus* Ag33 and *A. bitorquis* Ag4 (4). DNA samples were probed with the five plasmids listed in Table 1. These markers are located on five different chromosomes (16). DNAs of one set of subcultures from each of the 104 pairings were initially assayed with all five plasmid probes. Those samples showing heteroallelism at informative loci were considered to be sexually compatible. For each sample showing no heteroallelism at informative loci, DNA of a second subculture was assayed for heteroallelism. For each sample showing no heteroallelism in the second round of tests, a third subculture was assayed. Pairings showing no heteroallelism after three tests were listed as sexually incompatible.

Two homokaryotic offspring, s462 and s534, carried only Ag1-1 alleles at the five loci listed in Table 1. These RFLP markers were therefore uninformative in the pairings of s462 and s534 with Ag1-1. Instead, one homokaryon, s6, which carries the *A1* mating type (the Ag1-1 type), was paired with s462 and s534. In these two pairings, p33n25 was informative.

**Fruiting.** Spawn was prepared according to the method of San Antonio (33) with some modifications. Canning jars (500 ml in size), each containing 100 g of rye grain well mixed with 1.0 g of $CaCO_3$ and 120 ml of $H_2O$, were autoclaved at 15 lb/in$^2$ for 1 h. The lids of the jars were reversed to prevent sealing and to permit air exchange. After the mixture cooled, it was inoculated with a mixture of one offspring and one progenitor homokaryon. Mixtures of homokaryons were used in order to test, under approximately equal conditions, the fruiting ability of pairings from which no heterokaryons were recovered as well as pairings from which stable heterokaryons were recovered. For each offspring isolate, a 4-mm$^2$ slice of agar covered with mycelium was finely cut in 4 ml of liquid CYM, and 2 ml was mixed separately with each similarly prepared progenitor culture. Heterokaryon Ag93b, homokaryons Ag1-1 and Ag89-65, and a mixture of Ag1-1 and Ag89-65 were used as controls. Cultures were placed at room temperature (21 ± 1°C). Starting from the 3rd week, the cultures were shaken well once a week until the entire medium was well colonized. Fruiting was conducted at Sylvan Spawn Laboratory, Inc., according to standard commercial fruiting procedures (10). Compost was mixed with spawn and then covered with casing soil upon the full colonization of the compost. Three replicates were conducted for each backcross or control.

**Mating type assignment.** The formation of a stable heterokaryon was used as the sole criterion for compatible mating. RFLPs have been used as unambiguous markers for distinguishing homokaryons and heterokaryons in *A. bisporus* (5, 14, 22). In this study, the formation of a stable heterokaryon was characterized by the coexistence of both phenotypes of the paired homokaryons in equal signal intensities on the autoradiogram. The mating type of Ag1-1 was designated *A1*; that of Ag89-65 was designated *A2*. Therefore, homokaryotic offspring that form stable heterokaryons with Ag1-1 carry mating type *A2*, and those that form stable heterokaryons with Ag89-65 carry mating type *A1*. On the basis of this criterion, mating types of homokaryotic offspring were determined.

**Linkage analysis.** A series of pairwise $\chi^2$ tests of independent assortment between *MAT* and the other 64 loci were conducted as described by Kerrigan et al. (16). In these tests, expected numbers of parentals and recombinants were computed from observed allele ratios in the set of homokaryotic offspring for which both data were available. Once loci showing a significant deviation from the null hypothesis of independent assortment with *MAT* were identified, the order of *MAT* in relation to the other loci on its inferred linkage group (16) was determined with the TRY function of MAPMAKER MACINTOSH V1.0 (18). MAPMAKER was then used to calculate the maximum-likelihood map position of *MAT* by using the order found with the TRY function.

## RESULTS

**Mycelial interactions on petri plates.** Mycelial interactions on DCS medium were classified into three categories (Table 2). Among 104 backcrosses, 32 showed obvious fluffy growth along the junction zone, 24 showed a small amount of fluffy growth along the junction zone, and 48 showed no fluffy growth along the junction zone. No homokaryotic offspring showed positive mycelial interactions (obvious vigorous fluffy growth) in pairings with both progenitor homokaryons. Four homokaryons (s237, s298, s315, and s477) showed positive mycelial interactions with one progenitor and ambiguous mycelial interactions (a small amount of fluffy growth) with the other. Twenty-eight homokaryons showed positive mycelial interactions with one progenitor and negative interactions (no fluffy growth) with the other. Six homokaryons (s110, s369, s395, s401, s505, and s587) showed ambiguous mycelial interactions with both progenitors. Eight homokaryons (s45, s72, s166, s334, s448, s450, s542, and s673) showed ambiguous mycelial interactions with one progenitor and negative interactions with the other. Six homokaryons (s60, s78, s107,

TABLE 2. Results of backcrosses of 52 homokaryotic offspring to the homokaryotic progenitors Ag1-1 and Ag89-65

| Homokaryon | Ag1-1 | | | Ag89-65 | | | Mating type[d] |
|---|---|---|---|---|---|---|---|
| | Mycelial interaction[a] | Heterokaryosis[b] | Fruiting[c] | Mycelial interaction[a] | Heterokaryosis[b] | Fruiting[c] | |
| Ag1-1 | − | − | − | + | + | + | *A1* |
| Ag89-65 | + | + | + | − | − | − | *A2* |
| s6 | − | − | − | + | + | + | *A1* |
| s12 | + | + | − | − | − | − | *A2* |
| s45 | ± | + | − | − | − | − | *A2* |
| s60 | − | + | − | − | − | − | *A2* |
| s72 | ± | − | − | − | − | − | ? |
| s78 | − | − | − | − | + | − | *A1* |
| s83 | + | − | − | − | − | − | ? |
| s107 | − | − | − | − | − | − | ? |
| s110 | ± | − | − | ± | + | − | *A1* |
| s113 | − | + | − | − | − | − | *A2* |
| s129 | − | − | − | + | + | + | *A1* |
| s147 | − | + | − | − | − | − | *A2* |
| s166 | − | − | − | ± | + | − | *A1* |
| s184 | − | − | − | + | + | − | *A1* |
| s226 | + | + | − | − | − | − | *A2* |
| s234 | + | + | + | − | − | − | *A2* |
| s237 | + | + | − | ± | − | − | *A2* |
| s252 | − | − | − | + | + | − | *A1* |
| s254 | − | − | − | + | + | + | *A1* |
| s283 | − | − | − | + | − | − | ? |
| s286 | + | + | − | − | − | − | *A2* |
| s287 | − | − | − | + | + | − | *A1* |
| s298 | + | − | − | ± | − | − | ? |
| s315 | ± | − | − | + | + | + | *A1* |
| s334 | ± | + | − | − | − | − | *A2* |
| s352 | − | − | NT | + | − | − | ? |
| s360 | − | − | − | + | + | + | *A1* |
| s361 | − | − | − | + | + | + | *A1* |
| s369 | ± | + | NT | ± | − | − | *A2* |
| s395 | ± | − | − | ± | − | − | ? |
| s401 | ± | + | − | ± | − | − | *A2* |
| s426 | − | − | − | + | + | − | *A1* |
| s437 | + | + | − | − | − | − | *A2* |
| s448 | ± | + | − | − | − | − | *A2* |
| s450 | ± | + | − | − | − | − | *A2* |
| s460 | − | − | − | + | + | + | *A1* |
| s462 | + | +* | − | − | − | − | *A2* |
| s466 | + | + | − | − | − | − | *A2* |
| s477 | + | + | − | ± | − | − | *A2* |
| s492 | − | − | − | + | + | + | *A1* |
| s494 | − | − | − | + | + | − | *A1* |
| s500 | − | − | − | + | − | − | ? |
| s505 | ± | + | − | ± | − | − | *A2* |
| s513 | − | − | − | − | − | − | ? |
| s534 | + | +* | + | − | − | − | *A2* |
| s537 | + | + | − | − | − | − | *A2* |
| s542 | ± | + | − | − | − | − | *A2* |
| s587 | ± | − | − | ± | + | − | *A1* |
| s673 | ± | + | + | − | − | − | *A2* |
| s62p16 | + | + | + | − | − | − | *A2* |
| s88p49 | + | + | − | − | − | − | *A2* |
| s92p1 | − | − | − | + | + | + | *A1* |

[a] +, positive mycelial interaction; ±, ambiguous mycelial interaction; −, negative mycelial interaction.

[b] +, heteroallelism confirmed at all informative loci by RFLP analysis; −, no evidence of heteroallelism; *, results from the crosses with s6, which were confirmed by probe p33n25.

[c] +, fruit body or fruit body initial formed; −, no fruit body or fruit body initial formed; NT, not tested. Control heterokaryon Ag93b formed fruit bodies in this experiment.

[d] *A1*, homokaryon carries Ag1-1 mating type; *A2*, homokaryon carries Ag89-65 mating type; ?, mating type unknown.

s113, s147, and s513) did not show any mycelial interaction with either progenitor. Mycelial interactions for replicates were consistent for each pairing.

**RFLP analysis.** The results of the RFLP analyses are summarized in Table 2. None of the 52 homokaryotic offspring was found to form a stable heterokaryon with both progenitors. Forty-three were found to form stable heterokaryons with only one progenitor or, in the cases of s462 and

TABLE 3. Pairwise $\chi^2$ tests of random assortment between *MAT* and other selected loci on chromosome I

| Locus | Parentals[a] | Recombinants[a] | Recombination frequency | $\chi^2$ value | Significance[b] |
|---|---|---|---|---|---|
| *R21-3* | 7 + 22 | 1 + 10 | 0.28 | 5.2 | $P < 0.05$ |
| *R4-1* | 8 + 17 | 4 + 6 | 0.29 | 4.7 | $P < 0.05$ |
| *PEP1* | 15 + 15 | 10 + 3 | 0.30 | 7.7 | $P < 0.01$ |
| *R4-3* | 13 + 8 | 8 + 1 | 0.30 | 5.5 | $P < 0.05$ |
| *PEP2* | 15 + 18 | 7 + 3 | 0.23 | 12.5 | $P < 0.0001$ |
| *P33N25-11* | 14 + 17 | 8 + 4 | 0.28 | 8.5 | $P < 0.001$ |
| *P33N25-4* | 12 + 17 | 7 + 6 | 0.31 | 5.7 | $P < 0.05$ |
| *R18-6* | 5 + 11 | 10 + 11 | 0.57 | 1.0 | NS |
| *R9-2* | 7 + 10 | 7 + 7 | 0.45 | 0.2 | NS |
| *R4-4* | 9 + 7 | 6 + 7 | 0.45 | 0.3 | NS |
| *P33N25-9* | 13 + 8 | 15 + 5 | 0.49 | 0.2 | NS |

[a] Numbers of parental and recombinant offspring with respect to *MAT* and the marker listed to the left.

[b] The significance of deviation from the expectation shown. NS, not significant.

s534, with s6. Nine were found to form no stable heterokaryon with either progenitor. Among the 43 stable heterokaryons confirmed by RFLP analysis, 39 showed positive or ambiguous mycelial interactions when paired with the same progenitor with which heterokaryosis was confirmed (Table 2); in 7 of these cases (s110, s315, s369, s401, s477, s505, and s587), however, ambiguous mycelial interactions were also observed in the pairings with the other, presumably incompatible, progenitor. Four homokaryons (s60, s78, s113, and s147) were found to form stable heterokaryons with one progenitor, although no positive or ambiguous mycelial interactions with either progenitor were observed on petri plates. On the other hand, seven homokaryons (s72, s83, s283, s298, s352, s395, and s500), which were found to produce positive or ambiguous mycelial interactions in pairings with progenitors, produced no heterokaryons in any of three separate subcultures from the confrontation zones of each of the two backcrosses and were therefore considered to carry unknown mating types.

**Fruiting tests.** Thirteen crosses of homokaryotic offspring (s6, s129, s234, s254, s315, s360, s361, s460, s492, s534, s673, s62p16, and s92p1) with one or the other progenitor homokaryons formed fruit bodies or fruit body initials in compost culture (Table 2). The fruiting results were consistent with the data from mycelial interactions on the petri plates and from RFLP analyses. The remaining homokaryons failed to fruit in either backcross.

**Location of the mating type locus on the genetic map.** The mating types of 43 homokaryotic offspring are summarized in the mating type column in Table 2. The $\chi^2$ tests (Table 3) showed that *MAT* deviated significantly from random assortment with certain loci in the central region of chromosome I. The smallest recombination fraction between *MAT* and another locus was with *PEP2*. Interestingly, however, the TRY function of MAPMAKER consistently placed *MAT* on one or the other terminal region of chromosome I when various subsets of the markers in Table 3 were selected. With the full set of markers selected, *MAT* was placed adjacent to *R21-3*. This terminal placement was chosen because of the large numbers of double crossovers needed to account for *MAT* placed in any interior interval, especially those in the region of *PEP2*. The resulting maximum-likelihood location of *MAT* is on one terminus of chromosome I, 42.8 centimorgans (cM) from the closest marker, *R21-3* (Fig. 1). This map position of *MAT*, however, must be regarded as somewhat artificial (see Discussion). Nonetheless, we provide this placement here because it was arrived at by the same standard criteria used to construct the rest of the map (16).

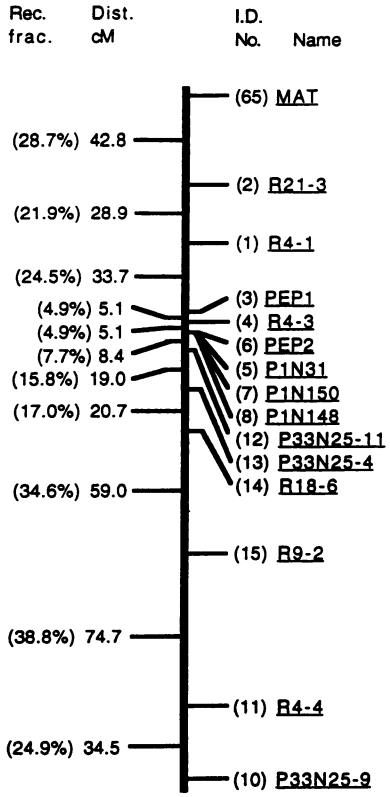


FIG. 1. A genetic map of chromosome I of *A. bisporus*. The position of the mating type gene *MAT* on the upper distal region was found with the TRY function of MAPMAKER V1.0. Distances between markers are depicted as the maximum-likelihood solutions provided by MAPMAKER V1.0. Parenthetical values on the left side indicate percentage of recombinants (recombinant fraction [rec. frac.]); adjacent values indicate map length (distance [dist.]) of intervals in centimorgans. Parenthetical numbers on the right side indicate code numbers for each locus. Detailed information regarding each locus on this map is provided by Kerrigan et al. (16). This placement of *MAT* should be interpreted as the best representation of the present genetic data. Larger numbers of homokaryotic offspring and the identification of a possible translocation affecting chromosome I could change the position of *MAT*.

## DISCUSSION

The mating type genes in basidiomycetes are important as switches in regulating sexual development (3, 11, 31, 36). This study describes and genetically maps the mating type locus in a segregating cross of the cultivated mushroom *A. bisporus*. The results of this study showed that each of the homokaryotic offspring mated with only one of the two progenitor homokaryons to form stable heterokaryons. The two mating types segregated in a ratio not significantly different from 1:1. Our results are consistent with the existence of a single segregating mating type locus in *A. bisporus*. We cannot rule out the possibility, however, that the mating type locus is complex. Analysis of larger samples of offspring might reveal novel recombinant mating types. For example, putative recombinant mating types have been

observed in the unifactorially heterothallic *A. bitorquis* (28). Also, in some bifactorial heterothallic basidiomycetes, such as *Schizophyllum commune* and *Coprinus cinereus*, intrafactor recombination in both *A* and *B* factors is found when offspring population sizes are sufficiently large (31, 36, 37).

In our assays, heteroallelism at informative loci assayed by RFLPs was interpreted as conclusive evidence of heterokaryosis and sexual compatibility. This criterion was successfully used to determine the mating types of 43 of the 52 offspring, with no heterokaryons detected in both backcrosses of a given offspring. There were, however, nine offspring for which no heterokaryons were detected in pairings with either of the two progenitors; in these cases, the mating types could not be assigned. We assume that each of these nine offspring is compatible with at least one of the two progenitor mating types. Failure to detect heterokaryosis in some compatible pairings might not be surprising in a backcrossed pedigree because of the possible exposure of recessive defects having a deleterious effect on heterokaryon formation or maintenance. For example, many possibly similar modifier mutations have been identified in *S. commune* (31). If a given inbred heterokaryotic genotype is homoallelic for recessive traits deleterious to heterokaryon formation or stability, then it will be less likely to be preserved through three rounds of subculturing and will be less likely to be detected by RFLP analysis.

Mycelial interactions among homokaryons of *A. bisporus* have been reported as being difficult to observe (7, 30). In our initial 104 backcrosses on CYM, we were able to observe only ambiguous mycelial interaction in a few backcrosses 6 to 7 weeks after inoculation. No positive mycelial interaction was observed. On the basis of another study of the effects of various media on mating interactions, we found that DCS medium was much more conducive to the expression of mating interactions than was CYM (unpublished data). Most of the mycelial interactions were clearly visible after 4 to 5 weeks of incubation on DCS medium. Although a visible mycelial interaction was generally associated with heterokaryosis in pairings, this association was not complete in the present study. For example, of the nine offspring producing no detectable heterokaryosis in pairings with either progenitor, seven showed at least some visible mycelial interaction in pairings with one or the other or both progenitors. Conversely, four homokaryons showed no visible mycelial interactions in pairings with either progenitor but nonetheless produced stable heterokaryons in pairings with one or the other progenitor homokaryon on the basis of RFLP analysis. Castle et al. (5) also found some cases in which macroscopic mating morphologies on petri plates were not concordant with RFLP results.

Why is the association of visible mycelial interactions and heterokaryosis in pairings in *A. bisporus* incomplete? The occasional absence of mycelial interactions from pairings producing heterokaryosis does not present a serious problem in interpreting mating behavior; stable heterokaryosis may not always be accompanied by a more vigorous aerial mycelium. However, the presence of visible mycelial interactions where no heterokaryons were detected, especially in pairings interpreted as incompatible, presents more of a problem in interpretation; apparently the visible mycelial interaction is not strictly confined to compatible pairings. It may be that complementation of recessive defects occurs in incompatible pairings through unstable nuclear associations, resulting in a small amount of fluffy aerial mycelium. Hyphal anastomosis is known to be constitutive in homobasidiomycetes, even in sexually incompatible pairings, and unstable associations of sexually incompatible nuclei are well known (31). Alternatively, there may be cross-feeding or syntrophy between adjacent hyphae, leading to a small amount of fluffy growth. In the absence of a firm explanation for the presence of mycelial interactions in incompatible pairings, we prefer to rely on stable heterokaryosis as the final arbiter of the outcome of matings of *A. bisporus*.

In addition to visible mycelial interactions, this study provides some limited information on the relationship of fruiting to mating type. Thirteen of the 52 homokaryotic offspring were found to form fruit bodies when crossed with one of the parental homokaryons. Each of these 13 pairings also produced heterokaryons in independent tests. Also, of all pairings for which no heterokaryons were detected, none fruited. While this observed fruiting is completely congruent with mating type assignments in Table 2, there were an additional 28 pairings deemed compatible on the basis of heterokaryon formation that did not fruit. There are two likely explanations for this high failure rate in pairings judged to be compatible. First, fruiting in *A. bisporus* is dependent on environmental factors (10). Since our spawn was not prepared in the same way as the standard commercial spawn, it may not have been optimal for fruiting. Second, backcrossing produces homoallelism at many loci, some of which may negatively affect fruiting. A similar effect has been reported in many crops (1) and in natural populations of some fungi (21). These two explanations are not mutually exclusive; both may have contributed to the high failure rate.

In spite of the high failure rate, however, the fact that all 13 pairings producing fruit bodies also showed positive mycelial interactions on petri plates and heterokaryosis, as verified by RFLP analysis, suggests that fruiting in *A. bisporus* is associated with mating. This association is not always evident in homobasidiomycetes. In the bifactorially heterothallic *S. commune*, homokaryotic fruiting can at times occur even when the mating type gene is "off" (12, 20, 31). In the present study, there is no evidence for homokaryotic fruiting; the two homokaryotic progenitors did not fruit, nor were there any cases of a homokaryotic offspring fruiting in both backcrosses with homokaryotic progenitors.

Having genetically identified the *MAT* locus of *A. bisporus*, we wished to place this locus on the existing genetic map (16). The *MAT* locus shows an unusual genetic behavior. Although the smallest recombination fraction between *MAT* and other loci is with *PEP2*, MAPMAKER places *MAT* on a terminus of chromosome I, a considerable distance from *PEP2*. This unexpected placement is due to the large numbers of double crossovers that must be invoked when *MAT* is placed on any of the interior intervals of chromosome I. This apparent contradiction could be easily explained by postulating the existence of heterozygosity for chromosome translocations in Ag93b. For example, in addition to producing duplication deficiencies (presumably lethal) in 50% of the total population of homokaryotic offspring, heterozygosity for a reciprocal translocation would also produce what appears to be a four-armed linkage map. If *MAT* is on a separate arm from the most central cluster of markers on chromosome I, then a large number of apparent double crossovers in the strictly linear map required by MAPMAKER would be generated by crossovers between *MAT* and the breakpoint of the translocation. The electrophoretic separation of chromosome-sized DNAs is entirely consistent with the hypothesis that translocations involving chromosome I are present in the Ag93b cross (32). We are currently testing this hypothesis by mapping chromosome I, including translocated segments, in detail.

One of our goals is to use genetic markers as an entry point to the physical isolation of *MAT* by chromosome walking. Unfortunately, the markers closest to *MAT* are located in the central region of chromosome I at about 25% recombination or about 35 cM away. What is the physical separation that corresponds to this genetic distance? The identified total genetic length of chromosome I is about 331 cM. The physical size of chromosome I estimated from clamped homogeneous electric field gels is 5.7 mb (32). If recombination frequencies are uniform along chromosome I (an assumption that may not be valid), then 1 cM would equal 17 kb. At this scale, the nearest markers would be about 600 kb away from *MAT*. Clearly a more complete map for chromosome I and translocated segments will be needed before chromosome walking can be initiated to isolate *MAT*. The further characterization of *MAT*, however, is desirable because it will allow us to better understand the regulatory mechanisms of development in *A. bisporus* and will also be useful to the mushroom industry in designing breeding strategies for strain improvement.

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