PRIMARY HOMOTHALLISM—RELATION TO HETEROTHALLISM IN THE REGULATION OF SEXUAL MORPHOGENESIS IN SISTOTREMA[1]

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ABSTRACT

The wood-rotting basidiomycete, *Sistotrema brinkmannii*, is an aggregate of biological species possessing several variations—homothallism, bipolar heterothallism and tetrapolar heterothallism—on the genetic regulation of a critical phase of development. Nutritionally forced intra- and interspecific matings provide genetic information about the relatedness of homothallic isolates, the relation of the various species to one another, the genetic basis of homothallism, and its relationship to heterothallism. Most homothallic isolates are interfertile when nutritionally forced. Successful hybridization between species is restricted to particular combinations of homothallic × bipolar isolates. Significant findings of these studies include: (1) documentation of hybridization of biological species in the Homobasidiomycetes, (2) documentation of the relatedness of two naturally occurring, variant systems, homothallism and bipolar heterothallism, that regulate sexual morphogenesis in the higher fungi, (3) evidence for definite, but limited, evolutionary divergence of the polygenic, regulated components of the respective systems, and (4) indication that the genetic basis of homothallism in this system is essentially due to constitutive function and consequently is fundamentally different from presently understood mechanisms in other self-fertile systems.

AMONG the higher fungi, the Homobasidiomycetidae, the conversion of the homokaryon into the dikaryon is termed "sexual morphogenesis". Typically, the homokaryon, a monosporous isolate or *strain*, is a mycelium with simple septa and uninucleate cells (nuclei haploid). The dikaryon is a mycelium with septal appendages termed clamp connections and binucleate cells with two haploid nuclei paired in each cell. The dikaryon is the fertile mycelium upon which sporulation occurs.

Four *patterns of sexuality*, bipolar and tetrapolar heterothallism and primary and secondary homothallism, have been defined on the basis of the manner in which sexual morphogenesis is genetically controlled. Heterothallic homokaryons must undergo mating interactions with compatible mates in order for sexual

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morphogenesis to occur. The mating type of a bipolar strain is determined by the particular allele that it possesses at a single gene or incompatibility factor, the *A* factor. Two bipolar mates are compatible if they possess different *A*-factor alleles. The mating type of a tetrapolar strain is specified by two incompatibility factors, *A* and *B*. Genetic studies in each of several tetrapolar species have revealed tetrapolar factors to be complex, i.e., each factor consisting of two linked loci or subunits, α and β. Four regulatory loci, $A\alpha$, $A\beta$, $B\alpha$, and $B\beta$, are thus involved in tetrapolar strains. Tetrapolar strains are compatible if they possess different mating types, i.e., differ in both *A* and *B* factors. The specificity of a tetrapolar factor is determined by the allelic constitution at its two loci, and allelic difference of the mates in either the α or β locus of a factor is sufficient for factor difference (RAPER 1966).

In contrast to heterothallic forms, homothallic forms are self-fertile. Monosporous isolates complete the life cycle in the absence of mating. Many of the species designated as homothallic possess the type of homothallism called secondary homothallism (WHITEHOUSE 1949). These parcel two compatible heterothallic nuclei (meiotic products) to each spore. Although this may be viewed as a genetically trivial avoidance of heterothallism, it may not be biologically trivial. On the other hand, *primary homothallism* (WHITEHOUSE 1949), in which spores are haploid and homokaryotic, is the less frequent and less understood case.

In bipolar and tetrapolar forms, the incompatibility factors control similar, if not identical, morphogenetic processes. Differentiation involves: the formation of fusion cells by two mates, and then, if the mates are compatible, the reciprocal exchange of nuclei, migration and proliferation of the exchanged nuclei within the pre-existing mycelia, nuclear pairing of migrant and resident nuclei in a 1:1 ratio within cells, and a complex synchronous process of nuclear and cell division involving clamp-connections.

Homothallic forms phenotypically mimic heterothallic homokaryons for a period subsequent to germination. Upon incubation, however, a spontaneous transition to the dikaryotic morphology takes place. Although both the primary and secondary homothallic forms are phenotypic dikaryons after the transition, OLIVE (1953) observed that the two are different in that the former is homokaryotic and the latter is heterokaryotic.

The genetic basis of apparent homothallism has been studied in several systems and shown to depend upon either of several distinct mechanisms. Secondary homothallism depends upon a mechanistic modification of heterothallism (SKOLKO 1944; SANSOME 1946; FRANKE 1962) due to the parcelling of compatible nuclei into individual spores. Two fundamentally different means of achieving self-fertility in basically heterothallic species have been described in yeasts. In species of the genus Saccharomyces, several investigations (WINGE and ROBERTS 1949; HERMAN and ROMAN 1966; TAKANO and OSHIMA 1967) suggest that self-fertility arises in clones of heterothallic cells through mutation of one mating type allele to the other (only two mating type alleles are known in Saccharomyces spp.). After the mutation occurs, compatible cells exist within the clone, and sexual interaction ensues. MATHIESON (1952) also suggested that mutation

of one mating type to the other in the filamentous ascomycete, *Chromocrea spinulosa*, accounts for apparent homothallism in that heterothallic species. LEUPOLD (1958) and GUTZ and DOE (1973) have suggested that homothallism occurs in the fission yeast, *Schizosaccharomyces pombe*, through the presence of two incompatibility loci approximately 1.1 map units apart. Homothallic isolates are thought to possess compatible mating type determinants at the two loci within a single genome.

In contrast, the genetic determination of primary homothallism and its relationship to heterothallism have never been adequately explained.

BIGGS (1973) reported the wood-rotting Homobasidiomycete, *Corticium coronilla*, synonym *Sistotrema brinkmannii*, to be an aggregation of species possessing homothallic, bipolar, and tetrapolar patterns of sexuality. In addition, BIGGS showed that two separate biological species exist among the tetrapolar forms of the aggregate. Later studies by LEMKE (1969) confirmed these findings and documented the presence of biological species within the homothallic and bipolar forms as well. Furthermore, LEMKE (1969) provided data proving the primary nature of homothallism in *S. brinkmannii*. This point has been confirmed and discussed by ULLRICH (1973).

The present studies were prompted by LEMKE's success (1966, 1969) in nutritionally forcing hybridization between a homothallic isolate and a bipolar strain. Eight viable progeny were recovered from this cross. Among these were homothallic isolates, bipolar isolates possessing the mating type of the bipolar progenitor, and a single self-sterile isolate apparently possessing a mating type differing from that of the bipolar progenitor. Other genetic markers discounted contamination. LEMKE (1969) interpreted these results as indicating that primary homothallic forms possess a cryptic or masked incompatibility factor analogous to the factors of heterothallic forms. It was thought that this masked or cryptic factor had been revealed by some recombinational event in the hybrid. The present study was undertaken to confirm and extend the findings of LEMKE, and to examine the relatedness of the biological species and the interrelationships of the variant patterns of sexuality.

MATERIALS AND METHODS

In an earlier study (ULLRICH 1973), stocks belonging to eight distinct groups within the *S. brinkmannii* aggregate were collected. Five biological species (biological in the sense of DOBZHANSKY 1937 and 1950 and MAYR 1940 and 1963—defined on the basis of reproductive isolation) were defined within the bipolar forms and two within the tetrapolar forms. A number of primary homothallic isolates were found as well. Auxotrophic mutations were induced in representatives of each of these groups by treatment of spores with nitrosoguanidine (ULLRICH 1973).

Several types of media were employed in this study. Two are complex: complete-plus-yeast (CYM—SNIDER and RAPER 1958) and malt extract (ME—NOBLES 1965); and two are defined: sporulation minimal (FM—LEMKE 1969) and minimal (MM—SNIDER and RAPER 1958).

Matings were nutritionally forced by inoculating two auxotrophic strains to a complete medium (*CYM* or *ME*), incubating until the isolates were in contact for as many as 7–21 days, and then transferring numerous plugs of inoculum to a minimal medium (*MM or FM*). These inocula were examined periodically for prototrophic growth at intervals up to two months.

Transfers to new minimal media were occasionally made. When prototrophic, putatively heterokaryotic growth was observed, transfers were individually established on minimal medium (FM) and periodically scored for fruiting. Monosporous isolates were established from the spores of mycelia that fruited. These isolates were scored for pattern of sexuality, nutritional markers, and mating type (if heterothallic). The patterns of sexuality possessed by the progeny were assigned on the basis of criteria as follows: isolate homothallic—clamp connections on the hyphae of a monosporous colony; isolate heterothallic—monosporous colony devoid of clamp connections, incompatible with heterothallic progenitor, and compatible with tester strains compatible with the heterothallic progenitor.

Three types of forced matings were studied. Initial forced matings of homothallic isolates were *intrastock*, the pairing of isolates derived from the same stock. These matings were made to demonstrate the feasibility of interacting homothallic isolates. A second group of forced matings involved homothallic isolates derived from different homothallic stocks (*interstock* mating). These matings were made to investigate whether homothallic forms represented a single intrafertile group or several intersterile groups. The third group of forced matings paired representatives of each pattern of sexuality and biological species—the eight distinct groups described above—in all possible combinations. Several representatives of each group were employed. In these matings, auxotrophic mutations served as genetic markers as well as forcing agents. The matings were made to examine the relatedness of the various groups and to obtain information about the genetic basis of primary homothallism and its relation to heterothallism.

RESULTS

Twenty-three combinations of homothallic intrastock matings involved auxotrophic strains derived from six different stocks. Eighteen combinations consistently produced vigorous prototrophic growth, one combination produced dense, but slow prototrophic growth, and four combinations produced no growth. Genetic analyses of progeny were made from six different intrastock matings. Despite the selection for prototrophic growth on minimal medium, there was a decided paucity of recombinant progeny and a skewed distribution among the progeny classes (cf. Table 1). Because doubly auxotrophic recombinants were absent among the progeny, an examination of reversion rates was made for each

TABLE 1

Nutritionally-forced intrastock matings of homothallic strains

Strains and forcing markers				Viability	Progeny classes of forcing markers				
Marker *a*			Marker *b*	%	a^+b^+	a^-b^+	a^+b^-	a^-b^-	Totals
33/3*	*met* ×	33/11	*ura*	100	0	59	0	0	59†
111/1	*ura* ×	111/6	*his*	100	0	199	18	0	217‡
111/6	*his* ×	111/9	*nic*	100	4	95	4	0	103‡
727/3	*his* ×	727/2	*ade*	100	6	189	3	0	198‡
143/3	*arg* ×	143/2	*lys*	50	0	15	0	0	15†
75/13	*ade* ×	75/18	*unk*§	100	0	177	[——18——]		195†

* 33/3 = strain #3 generated from stock #33.
† All progeny produced mycelia bearing clamp connections.
‡ Clamp connection status of progeny not scored.
§ unk = requirement unknown. N.B.: Due to the unknown nature of the requirement in 75/18 it is impossible to distinguish between progeny carrying a^+b^- or a^-b^+ without resorting to extended genetic analysis.

of the auxotrophic strains, in order to interpret the appearance of wild-type progeny. Revertants were found for strain 33/11 only.

Fertile prototrophic mycelia were common among homothallic interstock pairings. One hundred and thirty-five different pairings were made between auxotrophic strains derived from six homothallic stocks. At least one genetic analysis was made for each combination of progenitor stocks whose strains produced prototrophic, fertile mycelia. The results displayed in Table 2 demonstrate the feasibility of interstock homothallic matings and the role of meiosis in the fruiting process. The 1:1:1:1 ratios of the progeny classes for the recessive forcing markers rule out diploidy as the genetic mechanism behind primary homothallism in *S. brinkmannii*. Furthermore, the interfertility of these isolates suggests the homothallic forms to comprise a single interfertile group. This latter relationship is more clearly demonstrated in Figure 1 where interconnecting lines reveal the six stocks tested to form one continuous, intermating group. The figure also reveals a failure to mate in one of the 15 pairwise combinations of stocks, i.e., X-1-1 × 75. This failure may be attributable to the fact that only one auxotrophic isolate is currently available for stock X-1-1. In other interstock matings, certain combinations of strains are successful, whereas others are not. We have been unable to correlate these successes or failures with

TABLE 2

Nutritionally-forced interstock matings of homothallic strains

Stocks and strains designated as in previous table; —sh = reduced sulfur, i/v = isoleucineless-valineless.

Strains and forcing markers				Viability	Progeny classes of forcing markers				
Marker *a*			Marker *b*	%	a^+b^+	a^-b^+	a^+b^-	a^-b^-	Totals
33/13	*met* ×	X–1–1	*ura*	100	49	41	40	45	175
33/3	*met* ×	184/5	*i/v*	67	42	37	26	29	134
75/13	*his* ×	727/1	*lys*	100	47	49	58	43	197
75/13	*his* ×	184/5	*i/v*	54	23	29	18	12	82
111/1	*ura* ×	X–1–1	*ura*	100	12	[———	53	———]	65
111/1	*ura* ×	727/2	*ade*	100	63	73	72	61	269
33/13	*met* ×	75/13	*his*	74	8	13	10	6	37
33/11	*ura* ×	111/6	*his*	44	9	6	10	0	25
33/13	*met* ×	184/8	*nic*	50	7	10	7	6	30
33/11	*ura* ×	184/4	*—sh*	55	2	3	3	3	11
33/13	*met* ×	727/2	*ade*	68	9	17	7	1	34
75/13	*his* ×	184/8	*nic*	50	3	3	1	3	10
184/8	*nic* ×	727/3	*his*	68	11	8	11	4	34
184/8	*nic* ×	727/2	*ade*	45	1	1	1	6	9
184/5	*i/v* ×	727/1	*lys*	44	6	5	6	7	24
184/8	*nic* ×	X–1–1	*ura*	50	7	6	2	16	31
727/7	*ade* ×	X–1–1	*ura*	100	8	5	2	10	25*
75/3	*ade* ×	111/8	*arg*	100	3	2	15	11	31*
111/1	*ura* ×	184/8	*nic*	100	0	89	0	0	89*

* Clamp connection status not recorded; progeny of all other matings produced clamp connections.

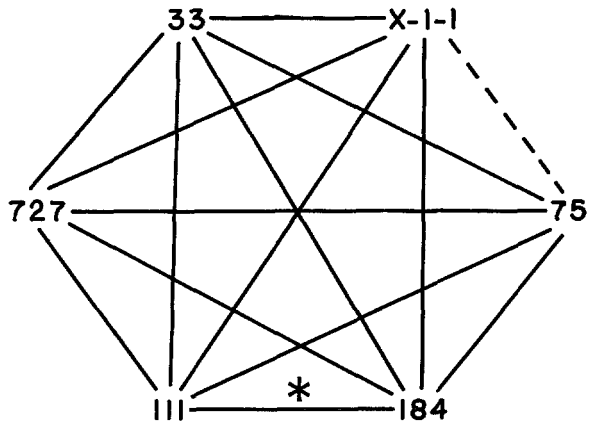

FIGURE 1.—Interfertility map of homothallic isolates. Numbers represent stock cultures. A solid line connecting two stocks indicates that strains derived from the two stocks have been observed to form fertile, prototrophic heterokaryons. A dashed line indicates the failure to produce a protophic mycelium.

* Prototrophic growth demonstrated, heterokaryosis not demonstrated.

any genetic character of the strains, e.g., particular nutritional deficiency; however, there is a consistent strain-dependent determination of the success or failure.

In Tables 1 and 2 the results of scoring the progeny for the presence or absence of clamp connections are indicated. In each case scored, clamp connections formed in about the same period of time as on selfed homothallic progeny. This point is significant to the interpretation of hybridizations.

Hybridization was exhaustively attempted in all pairwise combinations of auxotrophic isolates derived from the eight biological species listed above (5 bipolar, 2 tetrapolar, 1 homothallic). Successful hybridization was restricted to auxotrophic strains derived from a single homothallic stock (No. 75) force-mated with representatives of a particular bipolar species (that designated Group II-4 in earlier publications—ULLRICH 1973; ULLRICH and RAPER 1974). Success in hybridization is not dependent upon the particular mating type allele of the bipolar mate (cf. Table 3). Viability of hybrid spores is only 30% to 50%, whereas spores produced by "selfing" of the homothallic progenitor or by intraspecific mating of the bipolar parent have viability approximating 100%. Although the interfertility of descendants of one homothallic stock with isolates of one bipolar species reveals their relatedness, the reduced viability of hybrid progeny also indicates some evolutionary divergence. In each progeny class of the forcing markers (Table 3), the upper number indicates the total progeny and the lower number, in parentheses, the homothallic progeny. The P values for the chi-square goodness-of-fit of the forcing marker classes of progeny to the expected ratios of (1:1:1:1 or 1:3) are shown in the next-to-last column. For two of the four classes, there is good fit to the expectations, and for two others the fit is poor. Poor fit, however, is not unexpected in view of the depressed viability. Similarly, the P

TABLE 3

Genetic analyses of hybridizations between homothallic and bipolar heterothallic isolates
I = homothallic; II = bipolar heterothallic. Number of homothallic progeny in each class given in ( ), where analyzed.

Strains and forcing markers				Progeny classes of forcing markers					P values on χ^2 indicated	
I strain × II strain	Mating type of II mate	Viability %	a^+b^+ (I)	a^-b^+ (I)	a^+b^- (I)	a^-b^- (I)	Totals (I)	1:1:1:1 or 1:3‡	I:II 1:1§
Marker *a* Marker *b*									
75/4 *ade* × 932/5 *met*	A1	34	87 (44)	60 (20)	58 (18)	54 (27)	259* (109)	.05–.01	.05–.01
75/4 *ade* × 932/6 *ade*	A1	56	35 (20)	[——99——]			134* (73)	.70–.90	.30–.50
75/4 *ade* × 932/3 *his*	A1	47	42 (17)	14 (7)	41 (13)	17 (10)	114* (47)	≪.001	.05–.10
75/4 *ade* × 116/1 *ade*	A2	39	22 (12)	[——84——]			106* (52)	.30–.50	.90–.95
75/13 *his* × 932/6 *ade*	A1	41	3	10	0	4	17*		
75/4 *ade* × 116/6 *met*	A3	33	6	7	3	1	17*		
75/4 *ade* × 932/1 *his*	A4	11	4	4	1	2	11*		
75/4 *ade* × 932/2 *his*	A4	54	2	7	7	5	21*		
75/14 *ade* × 932/6 *ade*	A1	50	10	[——19——]			29*		
75/14 *ade* × 116/1 *ade*	A2	†							
75/13 *his* × 116/6 *met*	A3	†							
75/14 *ade* × 116/6 *met*	A3	†							

* Both recombinant classes present among progeny.
† Fertile, prototrophic mycelia derived; progeny analysis omitted.
‡ Auxotrophic markers.
§ Patterns of sexuality.

values obtained in the chi-square goodness-of-fit tests of a 1:1 expected segregation ratio of the homothallic pattern of sexuality to that of bipolarity (last column) suggest normal segregation in at least two of the four cases tested.

Each of 613 hybrid progeny analyzed for pattern of sexuality was eventually accommodated into a homothallic or bipolar pattern, and each of the 332 heterothallic progeny possessed the same mating type as its bipolar progenitor. In the mating interactions of some heterothallic progeny with wild, compatible, tester strains, dikaryosis was limited to small restricted areas. Some homothallic progeny were tardy and also restricted in attaining the dikaryotic phenotype in monosporous culture. Some of the retarded homothallic progeny required lengthy culture (up to six months on CYM at room temperature) in order to attain the dikaryotic phenotype. Cultures transferred at four months to medium with 1% glycerine replacing the glucose in MM quickly produced a few dikaryotic cells. By contrast, progeny from the mating of homothallic isolates or the selfing of homothallic stocks attained the dikaryotic state in 5 to 10 days on CYM at room temperature, and the entire mycelium of each was converted into a dikaryon. Curiously, hybrid homothallic products that are slow to attain the dikaryotic phenotype do so rapidly (1 to 2 days) when mated with tester strains from the bipolar biological species, II-4, regardless of mating type. This appears to be the

result of heterokaryosis, as conversion to the dikaryotic phenotype does not occur when the two mycelia are grown on opposite sides of a water-permeable membrane. Thus, the behavior of these tardy homothallic isolates nearly mimics the behavior expected of bipolar isolates possessing a mating type differing from that of the bipolar progenitor.

DISCUSSION

The intrastock forced pairings of homothallic isolates demonstrate the facility with which the mating of homothallic isolates may be accomplished. The skewed distribution of marker genotypes recovered among the progeny of intrastock matings (Table 1), however, was unexpected. Heterokaryotic cells appear to be rare in the intrastock matings, as evidenced by the paucity of recombinant genotypes among the progeny. A similar analysis of a single, forced, intrastock mating (LEMKE 1966, 1969) revealed a 1:1:1:1 segregation and assortment of two nutritional markers. Although the results of the present study cannot be reconciled with those of LEMKE, they must not be disregarded in view of the consistent, skewed distribution of the markers among the progeny in six of six matings constituted of strains from five different progenitor stocks. The absence of reversion in all but one of these mutant strains discounts the probability of explaining the wild-type progeny by reversion. Syntrophy or sectoring alone do not explain the low frequency of apparent recombinants. Also a disproportionate mixture of the amount of the homokaryotic mates is not sufficient to explain the results because some factor must be inhibiting, prohibiting, or destabilizing the existence of heterokaryotic cells in intrastock matings as opposed to interstock matings. Several precedents serve as possible explanations of the apparent rarity of heterokaryotic cells: imbalanced nuclear ratios (<10:1 to 4000:1) in the common-*A* heterokaryon of *S. commune* (RAPER and SAN ANTONIO 1954; SNIDER and RAPER 1965), and unstable heterokaryons in *Agaricus bisporus* (RAPER, RAPER and MILLER 1973) and *S. commune* (MIDDLETON 1962). This intriguing observation merits continued investigation because of the apparent effective disruption of heterokaryotic nuclear pairing in intrastock, as compared to interstock, matings of homothallic isolates.

The demonstrated interfertility of progeny of stock 33 with those of stock 75 differs from the earlier report of LEMKE (1969). This observation, plus the pattern of successful crosses of other homothallic isolates employed in the study, suggests that the homothallic forms of *S. brinkmannii* constitute a single interfertile group.

In contrast to the extremely skewed distribution in the progeny classes of the intrastock matings, the distribution in the interstock matings is quite equal. The last interstock mating in Table 2 is a conspicuous exception; however, the two progenitors (Nos. 111 and 184) were isolated only a few miles apart, and the matings may actually represent an interstock pairing.

Heterozygosity at various loci in interstock matings may enhance the frequency or stability of heterokaryotic cells and consequently account for the observed increase in recombinant progeny. Such a mechanism could promote outbreeding in the basically self-fertile system.

The restriction of successful hybridization to progeny of a single homothallic stock mated with bipolar strains of a particular biological species was unexpected. This success reveals the relatedness of some extant homothallic isolates to some bipolar isolates. The failure of other homothallic isolates to hybridize with bipolar isolates suggests genetic differences between homothallic forms with respect to this character. An extensive series of experiments is planned to genetically analyze this determinant by following the transmission of this ability to hybridize in progeny resulting from the cross of stock 75 with other homothallic isolates.

The other pairwise intergroup combinations of strains derived from the eight distinct biological groups failed to yield hybrids. The significance of this failure is presently obscure. Possible explanations include: (a) some of the groups may have diverged beyond the point of genetic compatibility even with nutritional forcing, and (b) the taxonomic criteria currently deemed critical may be wanting in that they "lump" forms exhibiting convergent evolution.

The present study of successful hybridization includes several bipolar strains possessing different mating types. The four most intensively analyzed matings (cf. Table 3), which include almost one hundred times as many progeny as in LEMKE's study (1969), fail to confirm LEMKE's preliminary results and interpretations concerning the genetic basis of primary homothallism and its relationship to heterothallism. In retrospect, it would appear that the earlier interpretation, based upon a single anomalous isolate, is subject to reinterpretation, as retarded homothallic isolates that readily mate with testers of the proper bipolar group mimic heterothallic strains possessing a different mating type. There is consequently little basis for the belief that homothallic forms possess an incompatibility factor (a) analogous to those of heterothallic forms, and (b) whose mating type is masked or made cryptic by some ancillary gene separable from the incompatibility factor by recombination.

The restricted expression of dikaryosis in matings between some heterothallic progeny of hybridization and wild heterothallic testers, as well as the delay and restriction in the attainment of the dikaryotic state in certain homothallic progeny of hybridization, support other evidence that homothallic forms have been genetically isolated from the bipolar species for a significant evolutionary period. Furthermore, the continuous variation noted in the degree of disturbance between various isolates suggests that this disturbance may be due to the recombination and assortment of a number of genes of the polygenic system. Most important, however, is the fact that the preponderance of hybrid progeny display little or no disturbance of the complex developmental phenomenon of sexual morphogenesis. This indicates that the genetic elements, both regulatory and regulated components, are fundamentally so similar that they usually function normally when recombined.

This study has revealed fundamental similarities between homothallism and bipolar heterothallism in *S. brinkmannii*. Earlier studies (LEMKE 1969; ULLRICH 1973) eliminated the possibility of diploid or binucleate heterokaryotic spores, and thus showed the homothallism to be primary. Homothallism could also be related to bipolar heterothallism by duplication of the incompatibility locus and inclusion of compatible alleles within the genome; however, neither aneuploidy

nor insertional translocations appear to be involved in *S. brinkmannii* (ULLRICH 1973). The results of homothallic × bipolar hybridization discussed here also discount aneuploidy or insertional translocation because of the absence of heterothallic progeny with a mating type differing from that of the bipolar progenitor. Conversion from heterothallism to homothallism by mutation of the mating type of one or more nuclei within a mycelium to a new mating type compatible with the pre-existing one also is improbable, as such mutations are unknown in this or related forms. Although the mutation of mating type has been documented in the conversion of heterothallic yeasts to self-fertility (cf. above), extensive mutagenic programs in the tetrapolar heterothallic basidiomycetes *Schizophyllum commune* or *Coprinus lagopus* or in the bipolar heterothallic basidiomycete *Polyporus palustris* have never produced mutant strains with new mating type specificities (RAPER and RAPER 1973; DAY 1963; FLEXER, personal communication). This negative finding obtains in spite of the strong selection for recovering such mutants that is inherent in the experimental system (demonstrated by reconstruction experiments).

These considerations reinforce the findings of the current study—the relatedness of certain homothallic and heterothallic forms, the absence of hybrid heterothallic progeny with mating types differing from their bipolar progenitor, and the 1:1 segregation of the genetic "determinant" of homothallism from the bipolar incompatibility factor—and suggest that primary homothallism may be an essentially constitutive system. Primary homothallic isolates may be analogous to certain mutant strains that can be produced in the laboratory (PARAG 1962; DAY 1963; RAPER, BOYD and RAPER 1965) by mutation of the incompatibility factor(s) of heterothallic species (*Schizophyllum commune* and *Coprinus lagopus*). These mutants are constitutive for those functions controlled by the incompatibility factor in which the mutation resides, and they consequently lack any identifiable mating type. An alternative hypothesis considers primary homothallic forms to arise through deletion of the entire bipolar incompatability locus. The genetic basis of primary homothallism is under continued investigation. It is already clear, however, that the genetic determination of homothallism in *S. brinkmannii* is fundamentally different from that of other self-fertile systems in which the genetic basis has been studied.

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