EDIBLE MUSHROOMS

Sexuality and Breeding

CARLENE A. RAPER

I. INTRODUCTION

T

Sexuality has been characterized for most of the cultivated edible fungi. Its nature defines the potentialities and limitations for developing new strains through breeding.

ISBN 0-12-168050-9

The majority of cultivated fungi belong to the most highly evolved group. All with a known pattern of sexuality are Basidiomycetes, and all but two are in the order Agaricales.

The earlier contributions to an understanding of sexuality in these and other fungi came primarily from incidental observations during studies on systematics and developmental life histories. A greater understanding has come more recently from concerted efforts to study fertility patterns in matings among progeny, critical cytological examinations throughout the life cycle, and genetic analyses.

II. BIOLOGICAL BACKGROUND

Fungi as a group exhibit an amazing spectrum of life cycles, patterns of sexual behavior, and mechanisms of sexual control. It is as if they were used as a testing ground for devices determining these basic biological features. The Basidiomycetes display a wide range in variation and this entire range is expressed among the cultivated forms.

The life cycle, pattern of sexuality, and sexual mechanism are the three parameters of sexual behavior. Life cycle defines the order and relative durations of successive developmental phases as they involve nuclear and morphological events. Sexual pattern is the nature of events leading specifically to fertility (fruiting, nuclear fusion, meiosis, and production of progeny); the progeny may be self-fertile or self-sterile and cross-fertile in particular combinations when mated with siblings. Sexual mechanism defines the genetic and morphological devices that control the sexual pattern. These three parameters vary independently within the limits possible for a given taxon.

A. Life Cycle

The typical life cycle of Basidiomycetes consists of nine steps which are illustrated in Figure 1 as follows: (1) Germination of a basidiospore initiates the development of (2), a haploid homokaryotic mycelium containing genetically identical nuclei and capable of indefinite independent propagation. The mycelium may or may not go through an asexual cycle via the production of oidia (spores formed as outgrowths of short hyphal branches) or chlamydospores (formed by the rounding up of hyphal cells). (3) Mating between two compatible homokaryotic mycelia through hyphal fusion (plasmogamy) establishes (4), the fertile mycelium, which is usually a specialized heterokaryon known as the dikaryon. The two sexually compatible and genetically dissimilar haploid nuclei of the mated

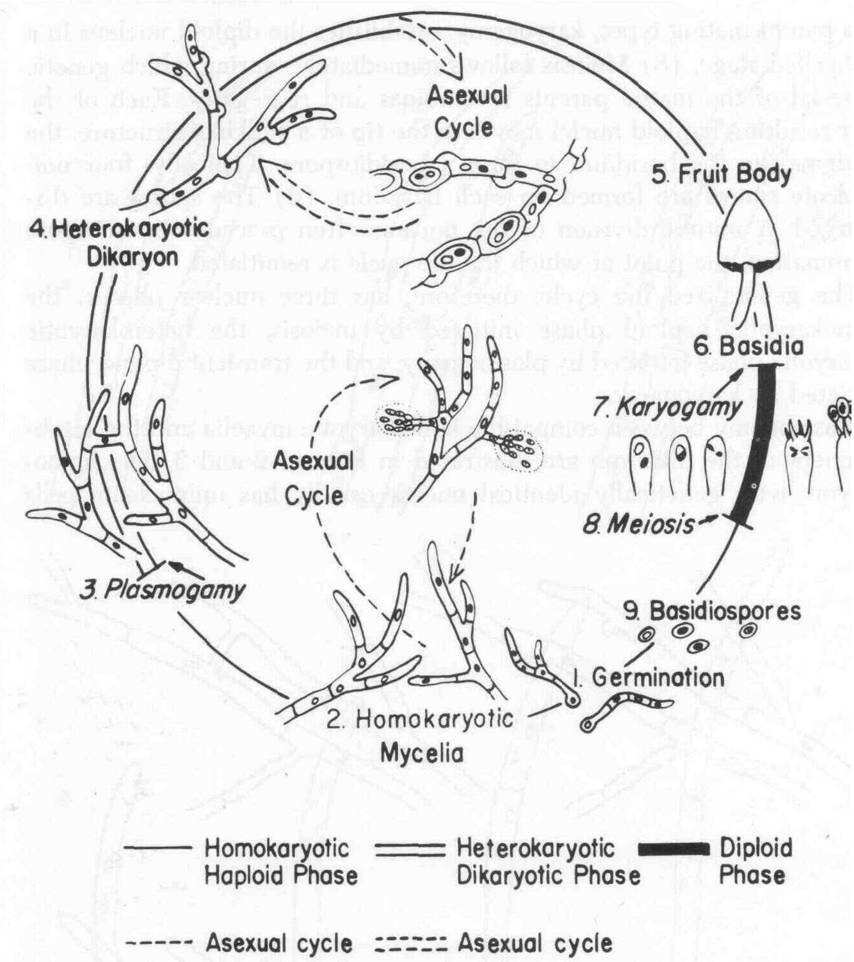

Fig. 1. Typical life cycle of Basidiomycetes.

parents are associated in pairs, one pair per cell, throughout the mycelium; each septum usually bears a bucklelike appendage called a clamp connection (see below). The dikaryon is capable of independent and indefinite propagation and may or may not go through an asexual cycle via the production of oidia or chlamydospores. If asexual spores are produced and are uninucleate, homokaryotic mycelia of the parental types are regenerated; if the asexual spores are binucleate, the dikaryon is regenerated. Under appropriate environmental influences the dikaryon produces (5), the fruit body as an outgrowth of specialized tissue. (6) The spore-bearing tissue of the fruit body develops as a columnar layer of club-shaped, binucleate cells termed the basidia. (7) Fusion of the paired nuclei of the

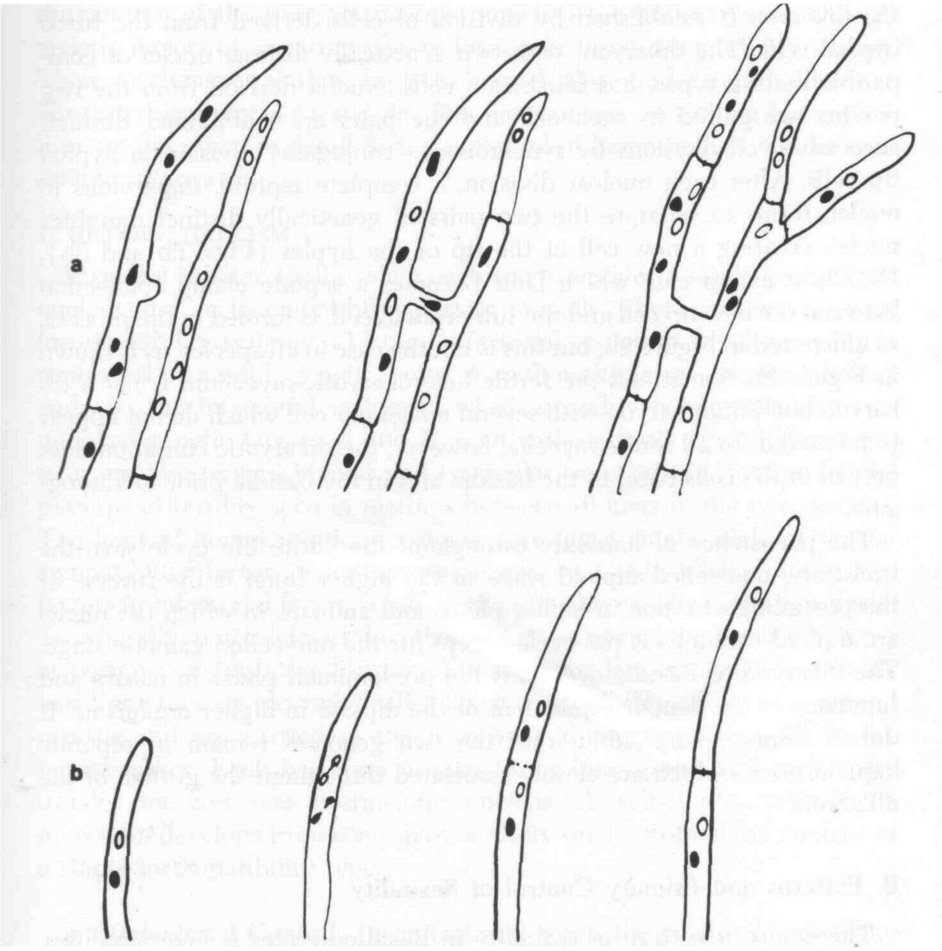

Fig. 3. Uncommon mating process in Basidiomycetes: (a) plasmogamy between compatible homokaryons with multikaryotic cells to establish the dikaryon; (b) conjugate nuclear division and cell division in a dikaryon without clamp connections. The black and white nuclei distinguish two compatible mating types.

and is known as a monokaryon (Fig. 2a), but it may be a multikaryon with several and various numbers of nuclei per cell (Fig. 3a). After plasmogamy between the hyphal elements of two genetically distinct homokaryotic mycelia of compatible mating types, the nuclei of the two mates become associated in a common cytoplasm (Figs. 2a and 3a). In most Basidiomycetes the invading nuclei migrate reciprocally into and throughout the existing mycelium of each mate to establish the heterokaryotic dikaryon. In a few species nuclear migration does not occur and the dikaryon is established by division of cells derived from the fused hyphal cells. The dikaryon, with two genetically distinct nuclei of compatible mating types, has binucleate cells. Nuclei derived from the two parents are paired in each cell and the pairs are maintained through successive cell divisions by synchronous (conjugate) division in hyphal tip cells. After each nuclear division, a complete septum, impervious to nuclei, forms to separate the two pairs of genetically distinct daughter nuclei, creating a new cell at the tip of the hypha (Figs. 2b and 3b). Usually a clamp cell, which later becomes a septate clamp connection between the new tip cell and the subterminal cell, is formed in the process, as illustrated in Figure 2b, but this is not the case in all species, as is shown in Figure 3b. Sometimes the fertile heterokaryotic mycelium is not a dikaryon but a multikaryon with several nuclei per cell which do not appear to be paired. In all fertile mycelia, however, the dikaryotic condition does prevail in the cells basal to the basidia and in the basidia prior to karyogamy.

The persistence of haploidy throughout the entire life cycle save the transitory, one-celled diploid stage in the higher fungi is the reverse of the prevailing situation in higher plants and animals, in which the nuclei are diploid throughout the cycle except for the one-celled gametic stage. The dikaryon of Basidiomycetes is the predominant phase in nature and functions as the genetic equivalent of the diploid in higher organisms. It differs from the diploid in that the two genomes remain in separate haploid nuclei which are closely associated throughout the growth of the dikaryon.

B. Patterns and Primary Control of Sexuality

The common pattern of sexuality in Basidiomycetes is heterothallism, in which plasmogamy in cross-mating between different homokaryotic mycelia (historically called thalli) is necessary to establish the fertile dikaryotic mycelium. (This is the illustrated pattern in Figs. 1, 2, and 3.) However, a variation with important consequences to breeding is imposed on the generalized life cycle by an alternate pattern of sexuality, known as homothallism, in which the mycelium (thallus) from a single germinated spore is self-fertile and has the competence to fruit in the absence of cross-mating. (Hyphal fusions occur both within and between homothallic mycelia, but they are not essential to the development of the fertile mycelium.) Homothallism is considered a sexual pattern because a sexual process, karyogamy and meiosis, occurs in the basidia of the fruit bodies of homothallic forms.

The patterns of sexuality are controlled by two mechanisms: (1) the distribution of the four postmeiotic nuclei to the basidiospores, and (2) genetic factors of a mating system known as the incompatibility system. These mechanisms as they apply to homothallism and heterothallism are illustrated in Figures 4a and 4b. The mechanisms controlling heterothallism are described in detail first; the control of homothallism will be considered afterwards.

1. HETEROTHALLISM

Sexuality is heterothallic when each spore receives a single postmeiotic nucleus and an incompatibility system prevails. There are two types of incompatibility systems: (1) the unifactorial system in which sexuality is controlled by a single genetic factor. *A*, with multiple specificities (alleles), and (2) the bifactorial system in which sexuality is controlled by two unlinked genetic factors, *A* and *B*, each with multiple alleles. These systems are also termed bipolar and tetrapolar, respectively, because of the patterns of fertility seen in matings between siblings in the two systems. The haploid homokaryotic mycelium, carrying a single allele of the incompatibility factor in unifactorial forms, or single alleles of the two factors in bifactorial forms, is self-sterile. Fertility results when two nuclei carrying different alleles of the relevant factors come together in a mating, or cross, to establish the dikaryon. The two nonidentical nuclei fuse in the basidium, then divide meiotically. The incompatibility alleles segregate at meiosis and are carried in the four haploid postmeiotic nuclei to the basidiospores. Each basidium usually forms four spores and each spore usually receives one postmeiotic nucleus. A self-sterile, cross-fertile mycelium develops from each spore with its single postmeiotic nucleus of a single incompatibility type.

a. Unifactorial Control. In unifactorial forms the single A factor controls compatibility as it relates to the entire sexual cycle. In a mating, a difference in *A* factor alleles (*A*) permits completion of the whole cycle. This typically involves migration of the invading nuclei into and throughout the resident mycelium—a reciprocal process in both mates—the establishment and propagation of the dikaryon accompanied by the formation of clamp connections between cells (true clamps), and ultimately the formation of fruit bodies on the dikaryon. The *A* factor appears to be a single genetic unit and, in a given cross, its two alleles, e.g., *A1* and *A2*, segregate in a ratio of 1:1. The usual four spores of the basidium represent a tetrad of which two carry one A allele and two carry the other A allele (Fig. 4b, lower right). The mycelia that develop from the four spores constitute two mating type groups and interact in a bipolar pattern when mated in all pairs (Table I).

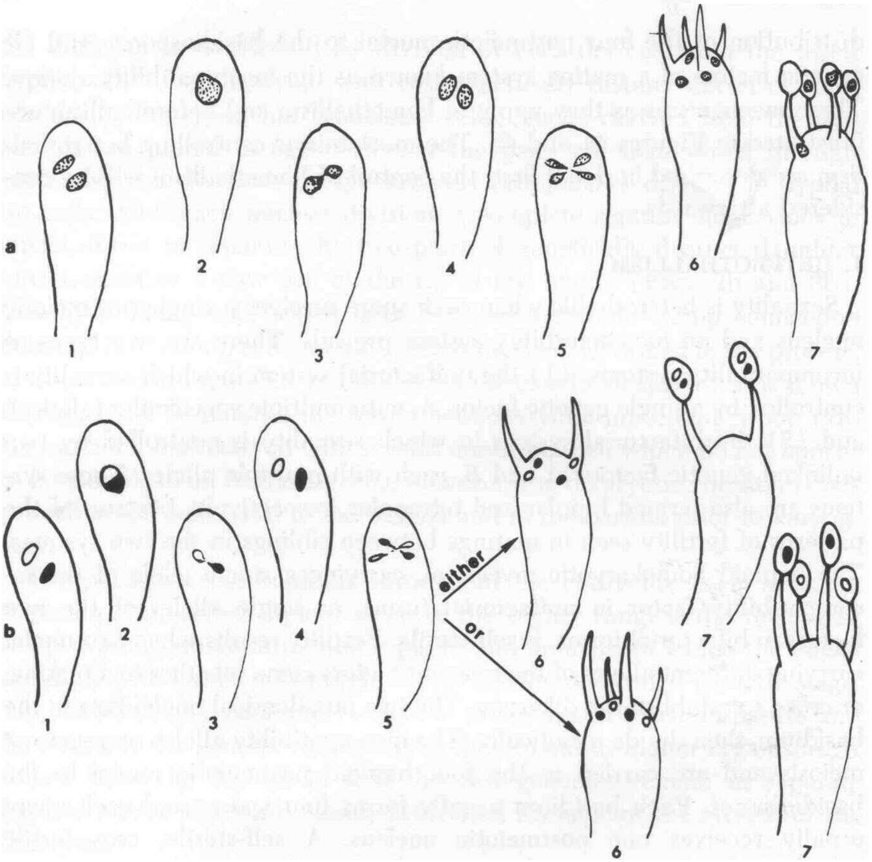

Fig. 4. The establishment of sexual patterns in Basidiomycetes. Karyogamy (1 and 2), meiosis (3, 4, and 5), formation of sterigmata and spores and distribution of postmeiotic nuclei to the spores on the basidium (6 and 7) are illustrated for three different patterns: (a) primary homothallism with no incompatibility factors (stippled nuclei), (b, upper right) unifactorial secondary homothallism with meiotic segregation of two alleles of a single incompatibility factor (black and white nuclei) and the inclusion of two compatible postmeiotic nuclei in each of two basidiospores, and (b, lower right) unifactorial heterothallism with meiotic segregation of two alleles of a single incompatibility factor (black and white nuclei) and the inclusion of a single postmeiotic nucleus in each of four basidiospores.

b, Bifactorial Control. In bifactorial forms the *A* and *B* factors have been shown to control distinct but coordinated parts of the sexual cycle. In a mating, the *A* factor controls nuclear pairing and clamp formation; the *B* factor controls nuclear migration and clamp fusion. A difference of alleles at the *A* factor but not at the *B* factor (*A* *B*=) is hemicompatible, involves no nuclear migration, and results in the formation of an infertile • *B*= heterokaryon composed of hyphae with dikaryotic tip cells but

TABLE I

The Bipolar Pattern of Sexual Interaction in a Typical Unifactorial Heterothallic Basidiomycete[a]

Incompatibility type		*A1*	*A1*	*A2*	*A2*
	Progeny number	1	2	3	4
A1	1	−	−	+	+
A1	2	−	−	+	+
A2	3	+	+	−	−
A2	4	+	+		−

[a] Mating interactions in all paired combinations are indicated for four progeny derived from the four spores of a basidium (a tetrad) from a cross involving the incompatibility types *A1* X *A*2. —, Signifies incompatibility and no interaction; +, signifies compatibility and complete interaction to produce a fertile dikaryon with true clamps. The siblings represent two groups with respect to mating type: all intra-group pairings are incompatible; all intergroup pairings are compatible.

monokaryotic subterminal cells and false clamps (the clamp cell fails to fuse with the subterminal cell, thus trapping one daughter nucleus after each division). The A *B*= heterokaryon, because of the lack of nuclear migration, is restricted to the zone of contact of the mated mycelia. A difference of alleles at the *B* factor but not at the *A* factor (*A*= *B*) is also hemicompatible, does involve nuclear migration, and results in the formation of an infertile *A*= *B* heterokaryon composed of hyphae with multikaryotic cells, with varied numbers of nuclei per cell, partial septa, and no clamps. The *A*= *B* heterokaryon, because of nuclear migration, is nonrestricted and develops throughout the mycelia of both mates. It is sometimes morphologically distinguishable from other heterokaryons and homokaryons by its paucity of aerial hyphae and generally poor, abnormal-appearing growth. It has been termed "flat" because of its depressed appearance. A difference of alleles at both factors (A *B*) is fully compatible and results in the formation of the nonrestricted, fertile, truly clamped dikaryon.

The *A* factor and *B* factor are not linked but segregate independently from one another. Hence the two parental *A-B* types segregate and recombine at meiosis to produce the four possible combinations of parental *A* and *B* types in a ratio of 1:1:1:1 in a sample of randomly collected progeny. For example, in the cross *A1 B1* X *A2 B2*, the four spores of the basidium may constitute a tetrad of the four incompatibility types *A1 B1*, A*1 B2*, *A2 B1*, and *A2 B2*. (Other possible tetrads are A*1 B1*, A*1 Bl*, *A2 B2*, *A2* B*2* or *A1 B2*, *A1 B2*, *A2 B1*, *A2 B1*.) The mycelia that develop from the four spores represent four mating type groups and interact in a tetrapolar pattern when mated in all pairs (Table II). A further detail of the bifactorial system must be considered: the incompatibility factors are not simple but complex. Each factor is constituted of two linked genes or loci: *A* and *A* in the *A* factor, and *B* and *B* in the *B* factor. The and genes of each factor are separable by crossing over; e.g., *A1* may actually be designated as *A* 1 1 and *A2* as *A* 2 2. A cross involving these two *A* types may yield a small percentage of nonparental, recombinant *A* types of two classes, *A* 1 2 and *A* 2 1, which may be designated *A*3 and *A*4, respectively. A difference of alleles of either gene, or , is sufficient for a compatible combination of the *A* types. The and genes of the *B* factor operate in a comparable way. In most bifactorial forms studied, the two genes of each incompatibility factor are closely linked and do not separate and recombine frequently. Therefore only the parental types are seen to segregate among the samples of progeny examined in most crosses.

Extensive multiple allelism for the incompatibility factors in both uni-

TAHLE **II**

The Tetrapolar Pattern of Sexual Interaction in a Typical Bifactorial Heterothallic Basidiomycete[a]

Incompatibility type		*A1 B1*	*A1 B2*	*A2 B1*	*A2 B2*
	Progeny number	1	2	3	4
*A*1*B*1	1	-	F	(+)	+
*A*1*B*2	2	F	-	+	(+)
*A*2*B*1	3	(+)	+		F
*A*2*B*2	4	+	(+)	F	—

[a] Mating interactions in all paired combinations are indicated for four progeny representing a four-type tetrad from a cross involving the incompatibility types *A*1 *B*1 X *A*2 *B*2. —, Signifies incompatibility; +, signifies full compatibility in which a nonrestricted fertile dikaryon with true clamps is formed; (+), signifies hemicompatibility in which an infertile restricted, principally monokaryotic heterokaryon with false clamps is formed. F, signifies hemicompatibility in which an infertile, "flat," nonrestricted and multikaryotic heterokaryon is formed. The siblings represent four mating types with the four possible combinations of parental *A* and *B* types. Each mating type is identified by its distinct set of interactions with the other three. In some species the hemicompatible interactions are not easily detected, in which case the tetrapolar pattern can be discerned on the basis of two criteria alone: full compatibility, +, versus incompatibility, —.

TABLE III

Pattern of Sexual Interaction Indicating Multiple Allelism for the Incompatibility Factors Found in Two Stocks of a Typical Bifactorial Heterothallic Basidiomycete

Incompatibility type	*A*3*B*3	*A*3*B4*	*A*4*B*3	*A4B4*
Stock 1				
*A*1*B*1	+	+	+	+
*A*1*B*2	+	+	+	+
*A*2*B*1	+	+	+	+
*A*2*B*2	+	+	+	+

[0] Mating interactions are indicated for progeny representing the four mating types of one stock (involving the incompatibility types *A*1, *A2* and *B*1, B2) with progeny representing the four mating types of another stock (involving the incompatibility types *A*3, *A4* and B3, *B*4). Complete compatibility, signified by +, in all matings, reflects the difference of alleles for both factors, *A* and *B*, in the two stocks.

factorial and bifactorial forms has been demonstrated by interstock matings in most species of Basidiomycetes studied. Compatibility or fertility patterns among mated progeny of fruit bodies collected in different areas have shown that the pair of segregating alleles for a given incompatibility factor of one such fruit body or stock are usually different from those of another (Table III).

2. PRIMARY HOMOTHALLISM

A homothallic pattern of sexuality in a Basidiomycete may be one of two types, primary or secondary. Primary homothallism, which has been established with certainty in only a few forms, does not appear to involve incompatibility factors. A self-fertile mycelium develops directly from a single spore with a single postmeiotic nucleus, and the presence of incompatibility factors cannot be detected (Fig. 4a). The fertile mycelium is homokaryotic, that is, has genetically identical nuclei. It may be dikaryotic, with or without clamps, but more often is multikaryotic without clamps. Karyogamy and meiosis do occur regularly in the basidia of the fruit body, but the heterokaryotic phase is missing in the life cycle of primary homothallic forms. Segregation and recombination of different genomes, therefore, does not normally occur. It can occur, however, on the rare occasions when one of the participating nuclei has, by chance, become mutated.

3. SECONDARY HOMOTHALLISM

Secondary homothallism has a distinctly different basis. It does involve incompatibility factors and is determined by the mechanism of nuclear distribution. The basidia of secondary homothallic forms usually bear only two spores each. Two compatible postmeiotic nuclei migrate into each basidiospore, and the self-fertile mycelium that develops directly from a single spore is intrinsically heteroallelic for the incompatibility factor or factors involved (Fig. 4b, upper right). The fertile mycelium is heterokaryotic, with two genetically different nuclear types. It is usually dikaryotic with clamps but may be dikaryotic without clamps or multikaryotic without clamps. The homokaryotic phase is missing in the life cycle of secondary homothallic forms. Secondary homothallic forms may be either unifactorial or bifactorial, and their functional distinctness from heterothallic forms is due only to the way in which the postmeiotic nuclei are regularly distributed in the basidiospores.

C. Secondary Control of Sexuality

A further dimension of the control of sexuality must be considered in the interpretation of fertility patterns among monosporous mycelia. The mechanisms of postmeiotic nuclear distribution in basidiospores and the genes of the incompatibility system are the primary controls of the sexual pattern, but environmental factors and genetic components other than the incompatibility factors impose a secondary level of control on sexual expression. Environmental conditions such as nutrients, temperature, and humidity have profound effects upon vigor of the potentially fertile mycelium and upon the fruiting process. Furthermore, many genes are involved in the various stages of sexual development, and defects in any or a few of these may block fruiting altogether. Sterility, therefore, cannot always be interpreted as a failure to satisfy the primary requirement for fertility, i.e., failure to include the appropriate combination of incompatibility types; it may sometimes be due to an inadequacy at the secondary level of control, such as a developmental mutant.

Comprehensive reviews of life cycles, sexual patterns, and sexual mechanisms in fungi are given by Whitehouse (1949), Raper (1960, 1966a), and Esser (1966). Sexuality in Basidiomycetes is discussed by Raper (1953, 1966b) and Koltin et al. (1972).

D. Consequences of Sexual Pattern

Heterothallism predominates in the Basidiomycetes. Of the few hundred species in which the pattern of sexuality has been worked out, about 65% are heterothallic under bifactorial control and 25% are heterothallic under unifactorial control. The remaining 10% are either primary or secondary homothallic forms (Raper, 1966b). The studied species, however, represent only about 10% of the estimated total of 5000 Basidiomycetous species. How well this proportion of heterothallism to homothallism reflects that of the natural population is not known.

Homothallism favors inbreeding and promotes homogeneity within the population; heterothallism favors outbreeding and promotes heterogeneity. The sexual pattern of a species and its consequences affect not only the adaptiveness of the species to its natural environment but also the success with which it can be handled in the laboratory for breeding purposes. The nature of sexuality, along with other basic characteristics such as growth requirements, spore germination, duration of life cycle, and fruiting competence, are known for most of the dozen or more cultivated fungi, and all of these features must be taken into consideration in the planning of an efficient breeding program for the development of desirable new strains.

III. SEXUALITY IN CULTIVATED FUNGI

Cultivated edible fungi are defined here as those fungi bearing fruit bodies, commonly termed mushrooms, that are particularly desirable as food in the marketplace and that can be cultivated in quantity, either in confined areas under precisely controlled conditions, or in wider outdoor areas under conditions manipulated by man to enhance mushroom production. Some 17 species of 11 genera of fungi are edible and cultivated. The extent to which these fungi are cultivated varies considerably. The most commonly known and widely produced mushroom is the long-domesticated *Agaricus bisporus* (Lange) Imbach. It is the predominant commercial mushroom of Europe and is virtually the only one produced and marketed in the United States. A wild species of the genus, *Agaricus bitorquis* (Quelet) Saccardo, is just beginning to be cultivated. Among the commercial mushrooms of Japan, the best known is the woodrotting shiitake, *Lentinus edodes* (Berk.) Sing., formerly known as *Cortinellus Berkeleyana* Ito et Imai. Others consumed principally in Japan are the gelatinous agarics *Pholiota nameko* (T. Ito) S. Ito et Imai and *Flammulina velutipes* (Fr.) Sing, (formerly known as *Collybia velutipes* (Fr.) Kummer), and the jellylike fungus *Tremella fuciformis* Berk. The jelly fungi of the genus *Auricularia, A. polytricha (*Mont.) Sacc., and *A. auricula-Judae (*Fr.) Schrot, are grown mainly in Taiwan and are included in many Chinese dishes. The fruit body of the latter fungus has a fancied resemblance to the human ear and is commonly called "Jew's ear," but the name *Auricularia auricula-Judae* is cumbersome and, as noted by Lloyd (1918), is a slander on the Jews. It has since become acceptable to drop *Judae* from the name, and this simpler nomenclature, *Auricularia auricula* (Hook.) Underw., will be used here. The most highly prized mushroom of Southeast Asia is the egg-shaped *Volvariella volvacea* (Fr.) Sing., the so-called paddy straw mushroom. Other mushrooms less commonly grown in Europe and Asia are *Stropharia rugosoannulata* Farlow apud Murr. and three species of the woodrotting fungus *Pleurotus, P. ostreatus* (Fr.) Kummer, *P. eryngii (*Fr.) Quel., and P. *cystidiosus* O. K. Miller. *Coprinus fimetarius* Fr., often confused with other related species, is delicate and highly perishable but can easily be cultivated. The truffle, most prized by epicures, is not a Basidiomycete but an Ascomycete and belongs to the order Tuberales. Three species of the genus *Tuber, T. melanosporum* Vitt., *T. uncinatum* Chatin, and *T. magnatum* (Pico) Vitt., are nurtured as mycorrhizal symbionts of the roots of oak trees in forests of southern France and Italy and are harvested with the aid of rooting pigs or dogs. The extreme high cost of these fungi reflect their low yield and difficulty of harvesting.

All of the cultivated fungi except truffles are Basidiomycetes and all but three of the Basidiomycetes are fleshy, gill-bearing fungi belonging to the order Agaricales. The exceptions, members of the genera *Tremella* and *Auricularia,* are Phragmobasidiomycetes bearing transversely septate basidia. Their jellylike, convoluted fruit bodies are characteristic of their order, Tremellales.

The nature of sexuality is not understood for the three species of the Ascomycetous truffles, nor is it known for the Basidiomycetes *Tremella fuciformis, Stropharia rugosoannulata,* and two of the *Pleurotvs* species, P. *eryngii* and *P. cystidiosus.** The life cycle, pattern of sexuality, and sexual mechanism have been characterized for the remaining ten cultivated species of Basidiomycetes, and they represent virtually every pattern of expression possible within the natural limits of this taxon. These defined species and their basic characteristics relevant to an understanding of life cycle and sexuality are given in Table IV.

A. *Volvariella volvacea*

Primary homthallism is represented by the cultivated subtropical mushroom *Volvariella volvacea* (Fr.) Sing., commonly known as the paddy straw mushroom. Characterization of sexuality is based on the self-

---

* See footnote on p. 107.

TABLE **IV**

Characteristics of Life Cycle **and Sexuality** in Cultivated Edible **Fungi**

			Homokaryon		Fertile heterokaryon		Asexual cycle		Sexuality	
Cultivated fungus	No. spores/ basidium	No. post-meiotic N./spore	No. N./cell	Fertile	No. N./cell	Clamps	Homokaryon	Heterokaryon	Incompat. factor(s)	Type
Volvanclla volvacea	4	1	many, variable	self-	—	—	yes	—	none	Primary homothallism
Agaricus bisporus	2	2	—	—	many, variable	no	—	no	*A*	Secondary homothallism
Agaricus bitorqnis	4	1	many, variable	cross-	2	no	no	no	*A*	Unifactorial heterothallism
Pholiota nameko	4	1	1?	cross-	2	yes	yes	yes	*A*	Unifactorial heterothallism
Auricularia auricula	4	1	1?	cross-?	2	yes	yes	no	*A*,?	Unifactorial heterothallism
Auricularia polytricha	4	1	1	cross-?	2	yes	yes	no?	*A*,*B*	Bifactorial heterothallism
Lentinus edodes	4	1	1	cross-	2	yes	no?	no?	*A*, B	Bifactorial heterothallism
Flammulina velutipes	4	1	1	cross-	2	yes	yes	yes	*A*,B	Bifactorial heterothallism
Pleurotus ostreatus	4	1	1	cross-	2	yes	yes	no	*A*, *B*	Bifactorial heterothallism
Coprinus fimetarius	4	1	1	cross-	9	yes	yes	yes	*A*, *B*	Bifactorial heterothallism

fertility of most monosporous isolates and the cytological evidence for meiosis in the basidium followed by the migration of one of the four postmeiotic nuclei to each of the four basidiospores (Chang and Chu, 1969, Chang and Ling, 1970; Chang and Yau, 1971; Chang, 1972). There is no evidence for the presence of an incompatibility factor (see Fig. 4a). Chang and Yau (1971) postulated an incompatibility factor, however, to explain the observed ratio of three self-fertile to one self-sterile progeny in the sample they studied. They suggested that self-fertility in the majority of the progeny resulted from the presence of an incompatibility factor composed of two linked genes, *A1* and *A2*, that operate together as two alleles of one factor. They further suggested that self-sterility in the minority of progeny resulted from unequal crossing over of these genes at meiosis to yield progeny with three incompatibility genes, *A*3 *A1 A1* or *A1 A2 A2*, and with a single gene, *A2* or *A1*. The fact that most of the self-sterile progeny produced fertile mycelia when mated with each other could be explained, they felt, by the pairing of unbalanced genotypes to provide the balance of incompatibility genes necessary for fertility. There is no evidence for such an incompatibility factor in *Volvariella volvacea*, nor is there any precedent in other fungi to suggest that an incompatibility factor could operate in this manner. An alternative explanation for the observed incidence of self-sterile, cross-fertile progeny in V. *volvacea* is a disruption by secondary genetic controls. The fact that self-sterility was usually coincident with aberrant mycelial morphology supports this view. The segregation of recessive mutant genes that alter morphology and block fruiting in a polygenic system could explain the observed phenomenon; there is precedence for this in other Basidiomycetes (J. R. Raper and Krongelb, 1958; C. A. Raper and Raper, 1966; Lemke, 1969; Perkins and Raper, 1970).

At present, the characteristics of the life cycle and of sexuality in *Volvariella volvacea—meiosis*, homokaryosis, and primary homothallism—are implied by cytological evidence and analysis of the fertility pattern. A genetic analysis in which mutant markers are traced through the life cycle has not been done in this species. Such an analysis could verify a sexual process, elucidate the major events of the life cycle and, perhaps, distinguish between alternative explanations for the incidence of self-sterility in this species.

In addition to the presumed sexual cycle, *Volvariella volvacea* has an asexual cycle via multinucleate chlamydospores. The vegetative spores are borne as spherical cells on specialized swollen cells of the multikaryotic mycelium. Clamp connections, common in the fertile mycelia of Basidiomycetes, are not present in this species (Chang, 1972).

B. *Agaricus bisporus*

The most widely cultivated fungus, *Agaricus bisporus* (Lange) Imbach, is the single example in this group of a secondary homothallic species. The self-fertility of monosporous mycelia has long been known (Lambert, 1929; Sinden, 1937; Klioushnikova, 1938; Sarazin, 1939; Kligman, 1943; Kneebone, 1968). Cytological evidence for meiosis in the basidium followed by migration of paired postmeiotic nuclei to each of two basidiospores provided the first hint of secondary homothallism (Sass, 1928; Colson, 1935; Sarazin, 1938; Evans, 1959; Jifi, 1965). The formation of two binucleate spores on each basidium is the normal pattern of development in this species, but occasionally a basidium may bear more spores. Analysis of fertility patterns among several monosporous isolates of rare four-spored basidia in *A. bisporus* supported the interpretation that homothallism in this species is of the secondary type; such isolates were found to be self-sterile and cross-fertile in a bipolar pattern (Miller, 1971; Miller and Kananen, 1972). Analysis of all four members of a tetrad of basidiospores also revealed self-sterility and cross-fertility in a bipolar pattern (Eliott, 1972). This pattern suggested control of fertility by two alleles of a single incompatibility factor *A*. In the usual pattern, each of the two spores that develops on the basidium receives two postmeiotic nuclei carrying different *A* alleles; each spore then develops into a self-fertile heterokaryon (see Fig. 4b, upper right). In the rare case when four spores form on the basidium, each receives one postmeiotic nucleus carrying a single *A* allele; each then develops into a self-sterile homokaryon capable of forming a fertile heterokaryon when cross-mated with another self-sterile homokaryon carrying a different *A* allele (see Fig. 4b, lower right).

Secondary homothallism in *Agaricus bisporus* has been substantiated by genetic analysis (Raper et al., 1972; Raper and Raper, 1972). Nutritionally deficient, auxotrophic mutations were obtained and used as genetic markers to trace the nuclear phases throughout the life cycle. Compatible nuclei carrying different *A* alleles and the marker genes were associated in stable, fertile heterokaryons. The markers were shown to segregate and recombine in what could best be interpreted as a meiotic process. The analysis provided strong evidence for a sexual cycle consisting of karyogamy and meiosis in the basidium followed by the inclusion of two compatible postmeiotic nuclei in each basidiospore and the development of a fertile heterokaryon with compatible haploid nuclei from each germinated spore.

The fertile heterokaryotic mycelium that normally develops from the germinated basidiospore is morphologically indistinguishable from the exceptionally occurring, self-sterile homokaryotic mycelium. The cells of both mycelia are multikaryotic and are also devoid of clamp connections. The creation of a fertile heterokaryon by crossing two compatible, self-sterile homokaryons can therefore be detected only through the tedious process of a test for fruiting. This fact, together with the extreme mechanical difficulty of isolating the rare self-sterile, cross-fertile homokaryons, presents serious problems to the breeding of this species.

The obstructions to breeding inherent in *Agaricus bisporus* can be circumvented by the mating of heterokaryons to obtain new heterokaryons under controlled, selective conditions. Crosses between self-fertile heterokaryotic strains do occur, but they are not normally recognized. They can be detected, however, by employing a technique known as nutritional selection. Heterokaryons homozygous for a recessive auxotrophic mutation can be mated in nutritionally complementing pairs, e.g., a heterokaryon requiring the growth substance adenine (*ade*-) but capable of making the growth substance proline (*pro*+) (the genotype *A1 ade*-~ *pro*+ + *A2 ade*$^-$ *pro*+) may be mated with a heterokaryon requiring proline (*pro*") but capable of making adenine *(ade+)* (the genotype *A1 ade*+ *pro*" + *A2 ade*$^+$ *pro*"). Neither heterokaryon alone can grow on a minimal medium devoid of its requirement, but a reassociation of nuclei in newly constituted compatible heterokaryons of the genotype *A1 ade*" *pro*+ + *A2 ade*+ *pro"* or the genotype *A1 ade*$^+$ *pro*- + *A2 ade*- *pro*+ will permit growth on the minimal medium through complementation: the genome of each nucleus provides the nutritional competence lacking in the other. Such matings are performed on a complete medium that supplies all requirements, including adenine and proline. Mycelium from the line of meeting of the mates is then subcultured on the minimal medium lacking these requirements; thus only a newly constituted, complementing heterokaryon capable of growth is selected. This is then fruited and progeny from the cross can be collected and analyzed. The secondary homothallic nature of *A. bisporus* makes the procedure of nutritional selection of heterokaryons virtually mandatory for the development of new strains through breeding in this species (Raper and Raper, 1972).

C. *Agaricus bitorquis*

Sexuality in the cultivatable wild species, *Agaricus bitorquis* (Qu£l.) Sacc., differs in an important respect from that in its domesticated relative, *Agaricus bisporus:* it is heterothallic rather than homothallic. Both species are unifactorial, with alleles of a single incompatibility factor, *A*, exerting the control; the significant difference in pattern of sexuality appears to be due to the difference in distribution of postmeiotic nuclei to the basidiospores. *Agaricus bitorquis,* like most members of the genus found in nature, is four- rather than two-spored. Cytological studies in several wild four-spored forms have indicated that each of the four spores of the basidium receives a single postmeiotic nucleus (Hughes, 1961; Jifi, 1965). This is in contrast to *A. bisporus* in which two postmeiotic nuclei are regularly included in each of the two spores of the basidium.

Heterothallism and the presence of a unifactorial incompatibility system have been revealed through a recent genetic analysis (Baper, 1976a,b; Raper and Kaye, 1978). Monosporous siblings were shown to be self-sterile and cross-fertile in a bipolar pattern. The fertile interaction in compatible matings was visibly distinguished by the development of a morphologically differentiated heterokaryotic mycelium in the zone of contact between the mates. The fertile heterokaryotic mycelium was whiter and had more aerial hyphae than the self-sterile homokaryotic mycelia of the mates. It was distinguished microscopically by its dikaryotic cells as compared to the multikaryotic cells of the homokaryons. No evidence of nuclear migration was apparent in the establishment of the dikaryon, and no clamp connections were seen (see Fig. 3). *A. bitorquis* was thus found to be not typical of other heterothallic Basidiomycetes; in most other such species studied, homokaryons are monokaryotic, nuclear migration does occur, and dikaryons bear clamps.

The segregation pattern of incompatibility alleles and nutritional genetic markers in this species confirmed the occurrence of meiosis, the uninucleate character of basidiospores, and their development into haploid homokaryotic mycelia. Eight collections from nature were studied and all proved to be completely interfertile. Several alleles of the *A* incompatibility factor were identified in the sample studied.

As in *Agaricus bisporus,* new prototrophic heterokaryons can be obtained by mating complementing auxotrophic heterokaryons, but the heterothallic nature of *A. bitorquis* makes this complicated process of nutritional selection unnecessary for performing crosses in a breeding program in this species.

D. *Pholiota nameko*

The cultivated Japanese mushroom *Pholiota nameko* (T. Ito) S. Ito et Imai is apparently heterothallic with unifactorial control (Arita and Takemaru, 1962). The basidium bears four spores and each spore is characteristically uninucleate, although a mitotic division has sometimes been observed following meiosis in the basidium and occasional binucleate spores are formed in consequence (Arita, 1973). Most germinated spores develop into homokaryotic, self-sterile mycelia with monokaryotic cells.

The fertile heterokaryon, developed in the mating of compatible homokaryotic mycelia, is a dikaryon with clamp connections. Arita and Takemaru analyzed the mating reactions among five collections of *P. nameko* and found multiple allelism for the *A* factor: nine of the ten *A* types in the sample were different from one another.

Both homokaryotic and dikaryotic mycelia have asexual cycles. The homokaryon has two types of asexual cycle, one through oidia and the other through basidiospores which form sparsely and erratically on relatively small, so-called homokaryotic fruit bodies in which basidiospore development presumably does not involve meiosis. Both types of spores germinate to regenerate the parent homokaryotic mycelium. The dikaryon also has an asexual cycle through the formation of both uninucleate and binucleate oidia. The uninucleate oidia develop into self-sterile homokaryotic mycelia of the two component mating types of the parent, a process known as dedikaryotization; the binucleate oidia regenerate the parent dikaryon (Arita, 1968).

E. *Auricularia auricula*

The earliest cytological observations on *Auricularia auricula* (Hook.) Underw. (Sappin-Trouffy, 1896) revealed the feature characteristic of Phragmobasidiomycetes: the basidium becomes four-celled by the formation of three transverse septa that separate the four postmeiotic nuclei. The four basidial cells remain attached and each develops a sterigma bearing a uninucleate spore. Upon discharge, each of the four spores germinates to produce a monokaryotic mycelium. The fruitbearing mycelium is presumably dikaryotic, although fruiting has not been explicitly correlated with dikaryosis.

From the single study of sexuality in this fungus Barnett (1937) designated the species as heterothallic with unifactorial control. He cultured monosporous isolates and observed their interactions in pairings between siblings. The monosporous mycelia bore no clamp connections. Matings between samples of siblings in all pairs revealed a bipolar pattern of interaction as diagnosed by the formation of clamped mycelia at the line of contact of the mates. The siblings could be divided into two groups with respect to their interactions: all pairings between groups produced clamped mycelia, and all pairings within groups did not. Barnett was unable to fruit this fungus reliably in the laboratory and therefore could not analyze the mycelia for fertility versus sterility, but he did show that at least one fruit-bearing mycelium was clamped. He did not mention nuclear migration—his matings were done in test tubes and samples of mycelia from only the zone of contact of the mates were removed from the tubes and examined—and he reported no cytological observations on the nuclear content of cells in undamped and clamped mycelia. Barnett's interpretation of unifactorial heterothallism in *A. auricula* is based solely on the pattern of occurrence of clamped mycelia in the restricted zone of contact in matings between siblings.

In the absence of information on fertility, nuclear migration, and nuclear content of cells, the alternative interpretation of bifactorial control cannot be ruled out in this species. It is not clear that all of the clamped mycelia developed in the pairing between members of the two designated mating type groups were uniformly dikaryotic and fertile, and it is not known to what extent, if any, nuclear migration was involved in these matings. Possibly the clamped mycelia seen to develop were actually of two types: (1) a nonrestricted fertile dikaryon with true clamps, and (2) a restricted, infertile heterokaryon with predominantly monokaryotic cells and false clamps. (A distinction between true clamps and false clamps is difficult to make without very thorough microscopic examination.) If the clamped mycelia were of these two types it would be necessary to invoke bifactorial control in this fungus: the fertile, truly clamped dikaryon would be established, possibly after nuclear migration, in a fully compatible *A* *B* mating, and the infertile, falsely clamped heterokaryon would be established without nuclear migration in a hemicompatible *A* *B*= mating. A distinction between bifactorial and unifactorial control of heterothallism in *A. auricula* will require further characterization of the nature and extent of the clamped mycelia developed in matings.

Multiple allelism for the incompatibility factor or factors was indicated by interactions between offspring representing the different mating types of several collections or stocks of *A. auricula:* the formation of clamped mycelia was observed in most matings between representative mating types of each stock studied (Barnett, 1937; Duncan and MacDonald, 1967). In matings between offspring of two of eight stocks, Barnett observed a low degree of compatibility that could not be attributed to the sharing of identical alleles of the incompatibility system. This phenomenon, probably involving genetic characters at the secondary level of control of sexuality, was confirmed in the later studies of Duncan and MacDonald. They examined interactions among 33 stocks of *A. auricula,* from Europe and North America and revealed what they termed three microevolutionary units. The European stocks were "intersterile" (did not produce clamped mycelia in matings) with the North American stocks, and two groups of North American stocks were partially "intersterile." Fruiting tests to distinguish between fertility and sterility were not done in this study. Duncan and MacDonald did, however, examine matings for evidence of nuclear migration and found none.

An asexual cycle, through the formation of clustered oidia, occurs in the monosporous, presumably homokaryotic, mycelium of this species.

No analysis involving the inheritance of genetic markers has been carried out in *A. auricula.* Such a study might help clarify answers to the questions that remain about sexuality in this fungus.

F. *Auricularia polytricha*

The nature of sexuality in the more commonly grown jelly fungus, *Auricularia polytricha* (Mont.) Sacc., appears to be heterothallic with bifactorial control (Su, 1973). Su found cytological evidence for meiosis and the formation of four uninucleate spores on the four-celled basidium of this species. He determined that monosporous isolates were monokaryotic without clamp connections. Pairings between siblings revealed a tetrapolar pattern of interaction as diagnosed by the formation of clamped mycelia. The clamped mycelia formed in the matings were restricted to the zone of contact but were of two types: dikaryotic with true clamps, and heterokaryotic with false clamps. No nuclear migration was observed, but the siblings could be divided into four groups with respect to their pattern of mating interactions: pairings within each group produced no clamped mycelium; pairings between members of each group and members of the other three groups resulted in four distinct patterns of interactions with respect to the formation of truly clamped dikaryons, falsely clamped heterokaryons, undamped multikaryons, and undamped monokaryons. This would be expected in a system with bifactorial control of heterothallism (see Table II). Presumably the dikaryon would be the only mycelium capable of fruiting, but fertility tests were not done in this study.

Evidence for multiple allelism of both incompatibility factors was revealed in analysis of several stocks (Duncan, 1972; Su, 1973). As in *A. auricula,* Duncan found indications of a microevolutionary process towards speciation in what he termed "partial sterility" between stocks of *A. polytricha.* Again, fruiting trials were not done to determine the extent of sterility or to analyze any genetic factors that might have been involved.

Su reported an asexual cycle through uninucleate oidia in the homokaryon of this species. He did not mention the existence of an asexual cycle in heterokaryons.

G. *Lentinus edodes*

Bifactorial heterothallism is clearly established in the shiitake, *Lentinus edodes (*Berk.) Sing. Careful cytological studies defined the fertile mycelium as clamped and dikaryotic; evidence for meiosis in the basidium and the subsequent development of four uninucleate spores on the basidium was also seen (Tanaka and Koga, 1972). The heterothallic nature of

Lentinus edodes has been revealed by several investigators (Oikawa, 1935; Nisikado and Yamauti, 1935; Takemaru, 1961a; Komatsu and Kimura, 1968a; Su, 1973). All characterized monosporous isolates as unclamped, self-sterile and cross-fertile. Su defined the monosporous, homokaryotic mycelium as monokaryotic. Oikawa, on the basis of the pattern of clamp formation in sibling pairings of 11 isolates from one stock, designated the species as heterothallic with unifactorial control. He sampled clamped mycelia only in the zone of contact between the mates and did not distinguish between true and false clamps. The authors of the other four studies, on the basis of larger samples more thoroughly examined, found a tetrapolar pattern of interaction among siblings and interpreted the control of heterothallism as bifactorial. Both Takemaru and Su made a point of determining the extent of the clamped mycelia developed in the matings analyzed and detected evidence of nuclear migration in the establishment of the dikaryon. *A* *B*= matings produced clamped mycelia only in the contact zone; *A* *B* matings produced clamped mycelia throughout both mates. Su noted that the restricted *A*= B= heterokaryon bore false clamps; he also detected the formation of nonrestricted *A*= *B* heterokaryons with multikaryotic cells. Fruiting competence, in addition to clamp formation, was analyzed in the studies of Nisikado and Yamauti, and of Komatsu and Kimura: only *A* *B* matings produced fertile dikaryons. Bifactorial control of heterothallism in this species has thus been demonstrated in many ways.

Multiple allelism for both incompatibility factors has been well documented (Oikawa, 1935; Takemaru, 1961a; Komatsu and Kimura, 1968a; Tokimoto *et al.*, 1973). All of these authors studied interfertility patterns in several stocks and identified several types of the A and *B* factors. Tokimoto *et al.* (1973) analyzed the largest sample. They identified 41 *A* types and 48 *B* types for the natural population of L. *edodes*. *A.* small percentage of nonparental *B* types was detected among the progeny of one stock (Takemaru, 1961a). These were interpreted as recombinants for the *B* factor of the two parents. By analogy to other bifactorial heterothallic forms studied, the *A* and *B* factors are each composed of two closely linked multiple-allelic genes capable of recombination to yield new factor types (Baper, 1966b).

Confirmation of a meiotic process was obtained by genetic analysis of mutant characters of the fruit body in *Lentinus edodes* (Komatsu and Kimura, 1964a,b, 1968b). Three recessive single-gene mutants, two for color and one for morphology, were each shown to segregate in a ratio of 1:1 with their respective wild-type alleles among the progeny of the relevant test crosses. They were not linked to the incompatibility factors, each of which also segregated in a ratio of 1:1 for the two parental types. These results are consistent with genetic segregation in a meiotic process and the subsequent production of haploid homokaryotic progeny, each derived from a spore with a single postmeiotic nucleus.

Asexual cycles are not mentioned in the literature on *Lentinus edodes.*

H. *Flammulina velutipes*

The voluminous literature on sexuality in *Flammulina velutipes* (Fr.) Sing, dates from as early as 1918, and the fungus is referred to throughout the literature by its former name *Collybia velutipes.* Its heterothallic character was first suggested by Kniep (1920) on the basis of his observation that unclamped mycelia develop from monosporous isolates and clamped mycelia develop from many germlings cultured together. Bifactorial control of heterothallism has been documented by many (Vandendries, 1923; Zattler, 1924; Heldmaier, 1929; Brodie, 1936; Oddoux, 1953; Aschan, 1954; Takemaru, 1957a, 1961b; Su, 1973). All of these investigators observed a tetrapolar pattern of clamp formation among samples of siblings mated in all paired combinations. The roles for incompatibility control by the two factors *A* and *B* (see Section II,B,1,b) were assigned arbitrarily and happened to be the reverse of those commonly designated for other bifactorial forms studied. This must be kept in mind when comparing the literature on sexuality in *F. velutipes* with that on other fungi with similar sexuality.

The fruiting competence of mycelia can be tested in laboratory cultures of this fungus. The homokaryon has been found to be generally sterile, although it may bear homokaryotic fruit bodies that are relatively small and produce few spores as compared to those of the fertile heterokaryon (Zattler, 1924; Heldmaier, 1929; Brodie, 1936; Aschan, 1954). Cytological studies revealed the homokaryon as monokaryotic and the fertile clamped heterokaryon as dikaryotic (Brodie, 1936; Takemaru, 1954; Su, 1973). Su observed also that the basidia are characteristically four-spored and the spores are generally uninucleate.

Evidence for nuclear migration in the establishment of the dikaryon in a fully compatible mating was seen by three of the investigators (Aschan, 1954; Takemaru, 1957a; Su, 1973). They noticed the formation of two types of clamped mycelia in matings, restricted and nonrestricted. Aschan and Takemaru noted also that the clamps of the restricted mycelia were true but did not occur at all septa. Su, on the other hand, observed that the clamps of the restricted and nonrestricted types were false and true respectively. In either case these differences could be correlated with hemicompatible and fully compatible combinations of *A* and *B* factor alleles in the matings. In addition, the *A*= *B* heterokaryon (by conventional terminology) was defined by Su as nonrestricted and multikaryotic.

Multiple allelism for the incompatibility factors was detected by analysis of interfertility patterns between stocks (Takemaru, 1961b). Both incompatibility factors were also shown to be composed of two linked genes, each of which has several alleles. Takemaru (1961b) proved the two-gene structure for one of the incompatibility factors in F. *velutipes* by identifying two classes of nonparental *B* types (his *B* factor was equivalent to the *A* factor of conventional terminology) in a single cross and regenerating the two original parental *B* types in a subsequent cross of the two nonparental *B* types. Becombination between the two genes of this factor to produce recombinant types ranged from 11 to *29%* in several crosses, thus indicating relatively loose linkage comparable to that known for the genes of the *A* factor in the woodrotting mushroom *Schizophyllum commune* (Baper et al., 1960).

Analysis of the inheritance of single-gene morphological mutants confirmed meiosis and the haploid homokaryotic nature of basidiospores (Takemaru, 1957b, 1961b).

Flammulina velutipes is one of the few Basidiomycetes in which an asexual cycle occurs in both homokaryotic and dikaryotic phases. Oidia are formed in chains on the hyphae. They are monokaryotic in homokaryons and either monokaryotic or dikaryotic in dikaryons. The interesting phenomenon of dedikaryotization through the isolation and culturing of uninucleate oidia from dikaryons has been studied quite extensively in this species. Evidence of cytoplasmic inheritance for some morphological differences between homokaryons before and after dikaryotic association was indicated by persistent differences in the morphology of homokaryotic cultures derived from monokaryotic oidia of dikaryons (Aschan-Aberg, 1960).

I. *Pleurotus ostreatus*

*Pleurotus ostreatus (*Fr.) Kummer, commonly called the oyster mushroom, is the only one of the several cultivated species of its genus with known sexuality.* Bifactorial heterothallism was first detected by Vandendries (1933) by analysis of clamp formation in a large sample of sibling

---

It has recently been noted that the sexuality of *Pleurotus cystidiosus* is probably known. *A*n article by S. C. Jong and J. T. Peng (1975), Identity and cultivation of a new commercial mushroom in Taiwan, *Mycologia* 67, 1235-1238, suggests a common identity for *Pleurotus cystidiosus* and *Pleurotus corticatus.* The latter was studied and described in an article by F. Kaufert (1936), The biology of *Pleurotus corticatus* Fries. *Minn. Agric. Exp. Stn., Stn. Bull.* 114, 35 pp. Kaufert described this species as heterothallic with bifactorial control. The main features of its life cycle appear to be the same as those of *Pleurotus ostreatus* with the exception that the dikaryon as well as the homokaryon has an asexual cycle.

pairings. His terminology for the incompatibility factors is different from that accepted by current convention; his *a, a'* and *b, b'* correspond to *B1, B2* and *A1, A2,* respectively (see Section II,B,l,b.) However, the tetrapolar pattern of interaction among siblings was clear. Terakawa (1957, 1960) confirmed these results and noted in addition the phenomenon of nuclear migration as it related to fully compatible and hemicompatible combinations of the incompatibility factors. (Terakawa's terminology also is reversed with respect to current convention; he refers to *A*'s as *B*'s and *B*'s as *A*'s.) Eugenio and Anderson (1968), using the conventional terminology, studied sexuality in this species most extensively. They found a tetrapolar pattern of interaction, including the formation of restricted mycelia with false clamps in *A* *B*= matings between siblings. Fruiting tests established the correlation between fertility and the truly clamped dikaryon formed in fully compatible matings. Su (1973) confirmed these findings with respect to sibling interaction and noted the multikaryotic characteristic of the *A*= *B* heterokaryon as well.

Cytological observations detected meiosis in the basidium, the subsequent formation of four uninucleate basidiospores, the monokaryotic characteristic of the homokaryon, and the truly clamped dikaryotic characteristic of the fertile heterokaryon (Terakawa, 1957; Su, 1973).

Genetic proof of meiosis and the haploid homokaryotic nature of basidiospores was obtained by analysis of the segregation pattern of a single-gene mutant, for fruit body color and the alleles of the incompatibility factors (Arita, 1974).

Multiple allelism for both incompatibility factors was first noted by Terakawa (1957) and amply demonstrated in a sample of over 20 dikaryons collected from nature by Eugenio and Anderson (1968). The latter investigators estimated from their data a total number of 63 *A* types and 190 *B* types in the natural world-wide population of this species. Nonparental incompatibility types were also seen to segregate in some crosses analyzed. They were presumably recombinants, but this was not proven.

/. *Coprinus fitnetarius*

Coprinus fimetarius Fr. is confused throughout the literature with several other species, most notably *C. lagopus,* but also *C. cinereus* and C. *radiatus* (Pinto-Lopes and Almeida, 1972). Nevertheless, sexuality in it and in the related species is comparable. This was one of the first fungi to be characterized with respect to sexuality, and genetical studies have been extensive.

Heterothallism was established with certainty by Bensaude (1917, 1918) when she identified monosporous cultures as sterile and monokaryotic and multisporous cultures as fertile and dikaryotic with clamps. Mounce (1922) was the first of several to provide evidence for bifactorial control of heterothallism on the basis of a tetrapolar pattern of interaction in sibling matings. She analyzed the matings for the production of clamped and fertile mycelia. Others confirmed this pattern (Brunswik, 1924; Hanna, 1925; Newton, 1926; Oort, 1930; Buller, 1931; Quintanilha, 1935). Hanna, Newton, and Quintanilha each detected segregation of four mating types in complete tetrads, and the latter investigator also identified false clamps on the restricted heterokaryon formed in *A*^= *B*= matings.

The phenomenon of mating between dikaryons and monokaryons was first demonstrated in *Coprinus* (Buller, 1931; Quintanilha, 1935), as was the phenomenon of septal degradation in the process of nuclear migration (Giesy and Day, 1965).

In analysis of interstock matings, both Brunswik (1924) and Hanna (1925) detected multiple allelism for the two incompatibility factors. On the basis of Brunswik's data, Whitehouse (1949) calculated a total of 27 *A* types and 27 *B* types in the natural population. A two-gene structure for the *A* factor was well established by Day (1959).

Meiosis in the basidium was cytologically documented (Lu, 1967) and thoroughly verified by detailed genetic studies (Day and Anderson, 1961). The genetic studies also confirmed the homokaryotic, haploid nature of basidiospores and defined two linkage groups with many genetic markers.

Both the *A*=T^ *B*= heterokaryon and the homokaryon are capable of producing underdeveloped fruit bodies. In addition, the homokaryon has an asexual cycle through the production of clustered uninucleate oidia (Bensaude, 1918; Buller, 1931). The dikaryon, which forms normal fruit bodies through a sexual process and fruits easily in laboratory culture, also has an asexual cycle through the formation of binucleate chlamydospores (Lewis, 1961).

IV. DISCUSSION

Our understanding of the basic biology of higher fungi has been significantly expanded by these studies of sexuality in cultivated edible fungi. Beyond this—and the sheer entertainment value of contemplating such variety of sexual expression as has been revealed—the knowledge obtained through these investigations has a sound practical value. It can be applied to the development of improved strains of cultivated fungi through breeding.

A. Breeding

Breeding is the only controllable means by which desired genetic traits of different stocks or strains can be combined. In the higher fungi, its minimal requirements are: a recognizable sexual interaction between mated strains, fruiting competence, meiosis, and viability of spores. The efficiency of the breeding process depends upon many additional features. An ideal candidate for breeding would have the following characteristics: heterothallic sexuality; a relatively short and predominantly haploid life cycle that can be completed in the laboratory under controlled conditions; spores that germinate quickly and in high incidence; defined and relatively simple requirements for culture; and a clearly distinguishable sexual interaction in which a fertile mycelium can be easily recognized, isolated, propagated, and fruited. This ideal fungus would also be found abundantly in nature and exhibit a wide range of variation in genetically determined traits, in order to provide ample choice of characteristics for breeding. An asexual cycle through oidia or chlamydospores would be relatively unimportant to breeding. The asexual spores might be disadvantageous as a source of cross-contamination in breeding experiments; they might, on the other hand, be advantageous in studying specific aspects of development, and also as a source of homogeneous, uninucleate cells in which to generate genetic markers with mutagenic agents.

Breeding is theoretically possible for all the cultivated fungi with a known sexual process. All can be fruited and go through meiosis. Spores are viable, although in varying degree. The life cycles are predominantly haploid and the vegetative phase in each can be propagated in pure culture on synthetic medium. Recognition of a sexual interaction has been demonstrated, with varying degrees of difficulty, in all of the fungi studied except *Volvariella volvacea,* and it may be possible to achieve this in the latter fungus as well.

The ease with which a breeding program can be carried out varies considerably among the fungi studied. It would be most efficient in the heterothallic forms. In fact, several of the heterothallic cultivated fungi approach the ideal in breeding potential. *Coprinus fimetarius, Pleurotus ostreatus,* and *Flammulina velutipes* seem most suitable, with their relatively short life cycles of three to six weeks and their easily distinguishable sexual interactions. *Agaricus bitorquis,* although somewhat handicapped by its longer life cycle of eight to ten weeks and its less easily distinguished sexual interaction, is also a good candidate for breeding. It is found abundantly in nature, has a variety of genetic traits for fruiting and mushroom morphology, and has already been bred for relatively high mushroom yields and a spore viability of greater than 90% (Raper, 1976b;

see also Chapter 15). *Pholiota nameko* also seems suitable for breeding according to the information available in the literature. *Lentinus edodes* is limited by the many months required for fruiting tests, and *Auricularia polytricha* and *Auricularia auricula* are also limited by the month-long period required for a sexual interaction that is not easily distinguishable.

The homothallic forms are far more difficult subjects for breeding. Breeding is possible in the secondary homothallic fungus *Agaricus bisporus* with the use of the nutritional selection techniques described for performing desired crosses between heterokaryons, but the procedure is cumbersome and time-consuming. It also appears that this species is not found abundantly in nature and the range of variation in genetic traits is not as wide as that for *Agaricus bitorquis.* A true sexual process has not yet been verified by genetic analysis in *Volvariella volvacea,* but on the assumption that this fungus is a primary homothallic form, as the present evidence suggests, breeding might be done by means similar to those described for *Agaricus bisporus.* Auxotrophic mutants would have to be obtained as a first requirement. If they could be used in the nutritional selection for heterokaryosis between strains and then could be shown to segregate in a meiotic pattern, breeding in V. *volvacea* would become a possibility. Conceivably, the self-sterile, cross-fertile isolates observed in the studies by Chang and Yau would also be used in a breeding program, but the blocks to fertility might prove to be a handicap in the long run.

B. Guidelines for Future Investigations

Breeding potential remains unknown for those cultivated fungi in which the nature of life cycle and sexuality has not been successfully investigated. Knowledge about one member of a genus, for example, *Pleurotus ostreatus,* is not specifically transferable to other members of the genus, such as P. *eryngii* and P. *cystidiosus.* A wide range of sexual expression, from both kinds of homothallism to both kinds of heterothallism, can exist among closely related species (Boidin, 1958; Lange, 1952; Lemke, 1969; Ullrich, 1973). Previous investigations of both cultivated and noncultivated Basidiomycetes have, however, provided guidelines for future studies on members of this class. They have described the general nature and specific parameters of life cycle and sexuality, and have also made available valuable methodology for studying these organisms. Future studies on those cultivated fungi not yet investigated might benefit most directly from previous studies on closely related species—for example, the study of *Stropharia rugosoannulata* from the previous work on *Stropharia semiglobata* (Oikawa, 1931), and the study of *Tremella fuciformis* from the previous work on *Tremella rubromaculata* (Furtado, 1968)—but the body of knowledge accumulated on Basidiomycetes in general provides a vital background for such studies.

The accumulated knowledge suggests the following guidelines for investigating those Basidiomycetes with unknown life cycles and unknown sexual characteristics:

1. Cytological examination for evidence of meiosis in the basidium, spore formation, and nuclear content of spores.
2. Germination of spores in pure culture and isolation of monosporous cultures.
3. Characterization of monosporous mycelia—macroscopic morphology, presence or absence of clamps, number of nuclei per cell, and fertility.
4. If monosporous mycelia are self-sterile, characterization of cross reactions by the mating of a sample of siblings in all pairs and the observation of any pattern of sexual interaction as indicated by type of mycelium developed in the matings—macroscopic morphology, presence or absence of true or false clamps, number of nuclei per cell, extent of heterokaryosis, and fertility.
5. Whether homothallism or heterothallism is indicated by previous steps, clarification of life cycle and sexuality through genetic analysis—the use of genetic markers, such as auxotrophic mutations and incompatibility alleles, to trace nuclear behavior throughout the life cycle.

The few edible fungi of commercial interest that are Ascomycetes, for example, the Tubers, require a different investigative approach. In contrast to Basidiomycetes, Ascomycetes develop their sexual spores in a saclike structure known as the ascus. The fertile dikaryon established in a mating is not propagated indefinitely but is restricted to ascogenous hyphae which give rise directly to ascal primordia within which karyogamy and meiosis occur. The ascogenous hyphae are completely dependent upon the haploid, homokaryotic mycelium of the maternal mate; hence, the tissue of the fruit body is primarily haploid and homokaryotic. The pattern of sexuality can be homothallism or heterothallism. Heterothallism is always unifactorial and the incompatibility factor has only two alleles, called *A* and *a*. The homokaryotic vegetative mycelium, which can be propagated indefinitely, is multikaryotic with septa permeable to nuclei. Multikaryotic heterokaryons that are capable of indefinite propagation and that may be heteroallelic for a variety of genetic markers are generally established by mating sexually incompatible mycelia, *A* X *A* or *a* X *a*- The establishment of such nonrestricted heterokaryons is precluded in sexually compatible matings, *A* X *a*, by the formation of the restricted dikaryon which is incapable of indefinite propagation. Compatibility with respect to the formation of nonrestricted heterokaryons between mycelia of like mating types is controlled by genes other than those of the sexual incompatibility system. Some of the methodology used for studying Basidiomycetes is obviously applicable to the study of Ascomycetes, but different procedures appropriate to the differences inherent in Ascomycetes, particularly as they relate to heterokaryosis, are also required.

C. Conclusion

The considerable background of information about the basic biology of the higher fungi that has been developed over the past decades should make its expansion relatively easy. With regard to edible fungi, both the cultivated forms and those with some prospects for cultivation, much remains to be added to that information already available. The application of such knowledge to breeding programs in commercially valuable mushrooms has just begun. Over the centuries of mushroom cultivation, far more attention has been given to methods of cultivation than to breeding in attempts to improve crop production. Yet the quality of the crop depends as much upon the inherited potential of the strain used as it does upon conditions for growth and development.

REFERENCES

Arita, I. (1968). Studies on the life cycle of *Pholiota nameko* (T. Ito) S. Ito et Imai. I. *Rep. Tottori Mycol. Inst.* 6, 49-57 (in Japanese with English summary).

Arita, I. (1973). Studies on the life cycle of *Pholiota nameko* (T. Ito) S. Ito et Imai. II. Karyological observations. *Rep. Tottori Mijcol. Inst.* 10, 383-388 (in Japanese with English summary).

Arita, I. (1974). Genetic study on white fruit bodies of *Pleurotus ostreatus* (Fr.) Kummer. *Rep. Tottori Mycol. Inst.* 11, 58-68 (in Japanese with English summary).

Arita, I., and Takemaru, T. (1962). Some problems on the mating system of *Pholiota nameko* (T. Ito) S. Ito et Imai. *Rep. Tottori Mycol. Inst. 2,* 1-10 (in Japanese with English summary).

Aschan, K. (1954). Some facts concerning the incompatibility groups, the dicaryotization and the fruit body production in *Collybia velutipes. Sven. Bot. Tidskr.* 48, 603-625.

Aschan-Aberg, K. (1960). Studies on dedicaryotization mycelia and of F_L variation in *Collybia velutipes. Sven. Bot. Tidskr.* 54, 311-328.

Barnett, H. L. (1937). Studies in the sexuality of the Heterobasidiae. *Mycologia* 29, 626-649.

Bensaude, M. (1917). Sur la sexualite chez les Champignons Basidiomycetes. *C. R. Hebd. Seances Acad.* Set. 165, 286-289.