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Biological Species Concepts in Eastern North American Populations of Lentinellus ursinus

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Biological species concepts in eastern North American populations of *Lentinellus ursinus*

(TITLE)

BY

Andrew N. Miller

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ABSTRACT

As part of a biosystematic study of the *Lentinellus ursinus* species complex, field and herbarium specimens from North America, Central America, and Europe were examined. Single basidiospore isolates from basidiomata collected in Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Tennessee, North Carolina, Wisconsin, Costa Rica, and Austria were crossed in all possible combinations to determine the mating system operating in each population. All populations exhibited bifactorial or tetrapolar mating systems. Tester strains were then crossed to identify intercompatible populations and to determine if morphological species in the *L. ursinus* species complex are congruent with biological species. Three biological species were recognized and identified as *L. ursinus*, *L. angustifolius*, and *L. vulpinus*. Polyspore isolates were subsequently used in culture mat and phenoloxidase analyses to facilitate identification of somatic cultures. The production of gloeocystidia and/or chlamydospores is taxonomically important for identifying somatic cultures of these species. All three taxa produced laccase, tyrosinase, and peroxidase in culture and were identified as white-rot fungi. This represents the first confirmed report of *L. angustifolius* from North America.

DEDICATION

To my mother and father

ACKNOWLEDGMENTS

For all of her patience, love and encouragement throughout this study, I thank my fiancee, Jennifer. I wish to thank my parents for their endless support, both emotionally and financially. None of this would have been possible if it were not for their love and encouragement.

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I. INTRODUCTION

The genus *Lentinellus* Karsten (Agaricales, Tricholomataceae) includes a group of pleurotoid fungi most commonly collected in late summer and fall in deciduous and coniferous forests throughout temperate regions. Although most species are found on decaying deciduous and coniferous wood, one species (*Lentinellus vulpinus* (Fr.) Kuhner & Maire) fruits on the wounds of living elm trees (*Ulmus* spp.) and another (*Lentinellus semivestitus* (Pk.) Singer) has been collected on dead grass roots (Miller and Stewart, 1971).

Although classical taxonomy has clearly delineated *Lentinellus ursinus* (Fr.) Kuhner from *L. vulpinus*, it has not accounted for additional cryptic taxa within the *L. ursinus* species complex and reveals nothing about reproductive compatibility between populations. Since preliminary data suggested that more than one biological species was represented in the *L. ursinus* species complex in North America, mating studies were proposed to identify sexually compatible and incompatible populations (i.e., biological species) in the *L. ursinus* species complex in eastern North America.

The hypothesis of this thesis is that more than one biological species occurs in the *L. ursinus* species complex in eastern North America. The objectives were to: 1) determine the mating systems operating in this species complex; 2) identify sexually compatible and incompatible populations; 3) determine the taxonomic value of culture mat analyses and phenoloxidase

reactions; and, 4) identify unique morphological features which can be used to segregate taxa in this species complex.

A. Taxonomic and Nomenclatural History

Fries (1821) initially included all fungi which produced lamellate basidiocarps in one of several tribes within the genus *Agaricus*. Within *Agaricus*, Fries (1821) included the following species which were later referred to *Lentinellus*: *A. cochleatus* Fr. (Tribe Omphalodus), *A. flabelliformis* Bolt: Fr. (Tribe Pleurotus), *A. ursinus* Fr. (Tribe Pleurotus), and *A. vulpinus* Sow: Fr. (Tribe Crepidotus). In 1825 Fries proposed a new genus, *Lentinus*, to include taxa with leathery-pliant pilei, entire or toothed lamellar margins, and white basidiospores. Fries (1825) transferred 20 taxa from *Agaricus* into *Lentinus* including the following species which were later referred to *Lentinellus*: *L. cochleatus* (Fr.) Fr., *L. flabelliformis* (Bolt.: Fr.) Fr., and *L. ursinus* (Fr.) Fr. Later, Fries (1838) transferred *A. vulpinus* to *Lentinus* and proposed a new species, *L. castoreus* Fr. In 1863 Fries described a final new taxon, *L. cochleatus* var. *omphalodes* Fr.

Karsten (1879) later proposed the genera *Lentinellus* and *Hemicybe* to include some of the taxa previously included in *Lentinus sensu* Fries. In *Lentinellus* Karsten (1879) included *L. cochleatus* (Fr.) Karst., *L. friabilis* (Fr.) Karst., *L. omphalodes* (Fr.) Karst, and *L. umbellatus* (Fr.) Karst. In *Hemicybe* Karsten (1879) placed *H. auricula* (Fr.) Karst., *H. castorea* (Fr.) Karst., *H.*

flabelliformis (Bolt: Fr.) Karst., *H. martianoffiana* (Kalchbr.) Karst., *H. svavissima* (Fr.) Karst., *H. ursina* (Fr.) Karst., and *H. vulpina* (Sow: Fr.) Karst.

Without citing Karsten (1879), Fayod (1889) transferred taxa in the *Lentinus cochleatus* group, distinguished microscopically by a parallel lamellar trama, cystidia with rounded apices, and spherical, granular spores to *Lentinellus sensu* Fayod although this generic name was previously occupied (Karsten, 1879). Kuhner and Maire (1934) accepted Fayod's concept of the genus and added an additional microscopic characteristic to the generic circumscription; the amyloid reaction of the basidiospores. Pilat (1941) subsequently transferred taxa with rough basidiospores to *Lentinaria* while maintaining taxa with smooth basidiospores in *Lentinellus*. Singer (1962) considered *Hemicybe* Karst., *Lentinellus* Fayod, and *Lentinaria* Pilat to be synonyms of *Lentinellus* Karst. Miller and Stewart (1971) accepted *Lentinellus sensu* Karsten and described the macro- and micromorphology of *L. cochleatus* (Fr.) Karst., *L. flabelliformis* (Bolt: Fr.) Ito, *L. montanus* O.K. Miller, *L. omphalodes* (Fr.) Karst., *L. pilatii* Herink, *L. semivestitus* (Pk.) Singer, *L. ursinus* (Fr.) Kuhner, and *L. vulpinus* (Sow: Fr.) Kuhner & Maire in their treatment of primarily North American species of *Lentinellus*. Finally, Pegler (1983) reviewed the status of all currently known species of *Lentinellus* in his monograph of *Lentinus*, and Stalpers (1996) suggested as many as 14 taxa may occur worldwide.

In addition to differences in disposition of taxa in *Lentinellus*, there has also been considerable debate as to which family *Lentinellus* belongs. Although

Singer (1943) included *Lentinellus* in the family Tricholomataceae, he (Singer, 1962) later suggested combining *Lentinellus* with aphyllophoraceous genera such as *Auriscalpium*, *Echinodontium*, and *Hericium* into a single family based on similarities in basidiospore morphology and reaction of gloeocystidia in sulfobenzaldehyde (Kuhner and Romagnesi, 1953). Maas Geesteranus (1963) later proposed the family Auriscalpiaceae to include *Auriscalpium*, *Gloiodon*, and *Lentinellus*, based on affinities he found between *Auriscalpium villipes* (Lloyd) Snell & Dick, *A. vulgare* S.F. Gray, *Lentinellus cochleatus*, and *L. ursinus*. Kotlaba and Pouzar (1972), who believed it was necessary to separate a lamellate genus (*Lentinellus*) from non-lamellate genera, proposed the family Lentinellaceae to include *Lentinellus*. Julich (1981) accepted Kotlaba and Pouzar's (1972) designation of the Lentinellaceae and placed this family in the Hericiales *sensu* Julich, which includes genera with smooth to warted, amyloid basidiospores, mono- or dimitic hyphal systems, and gloeoperous hyphae. Stalpers (1996) included *Lentinellus* in his keys to the species of the Hericiales.

B. Generic Description and Disposition of Taxa

Basidiomata clitocyboid (Largent, 1977) to pleurotoid in stature, tough, relatively long-lived, development gymnocarpous. In profile, pileus convex to infundibuliform in stipitate forms, conchate to plane in sessile forms. From above, pileus orbicular to flabelliform in stipitate forms, dimidiate to flabelliform in sessile forms. Pileus surface glabrous, tomentose or partially hispid, buff to

reddish-brown or blackish-brown. Context white to buff, fibrous, unchanging on exposure. Lamellae serrate to serrulate, broad, pale ochraceous to brownish (Stalpers, 1996). Stipe central, eccentric, lateral or absent, surface glabrous to tomentose, concolorous with pileus. Pileipellis a cutis of radially arranged, thin-walled, filamentous hyphae that appear cellular in cross section (e.g., *textura angularis* of Miller and Stewart, 1971). Pileocystidia, if present, thin- to slightly thick-walled, solitary or in fascicles. Basidia clavate, thin-walled, four-sterigmate. Basidiospores white in mass, globose to broadly ellipsoid, echinulate, thin-walled, amyloid. Hyphal system monomitic, composed of thin- to thick-walled, clamped, generative hyphae, hyaline to yellow in NH_4OH, thick-walled hyphae sometimes amyloid. Trama of many taxa (Stalpers, 1996) with thin-walled gloeoplerous hyphae (e.g., oleiferous hyphae of Singer (1962)) which originate in the subhymenium and terminate as gloeocystidia (e.g., macrocystidia of Romagnesi (1944); pseudocystidia of Singer (1962)). Gloeoplerous hyphae (e.g. oleiferous hyphae of Singer (1962) and Largent et. al. (1977)) with resinous contents, produce stearyl-velutinal (Camazine et. al., 1983), stain dark purple in sulfobenzaldehyde.

C. *Lentinellus* in Relation to Other Genera

Lentinus Fr. -- *Lentinus* is most readily separated from *Lentinellus* by smooth, ellipsoid, inamyloid basidiospores (Miller and Stewart, 1971). *Lentinellus* has echinulate, globose to broadly ellipsoid, amyloid basidiospores.

In addition, species of *Lentinellus* are white-rotters whose cultures produce a positive reaction with guaiacol and distinctive staining reactions on gallic and tannic acid agars. Species of *Lentinus* are brown-rotters whose cultures do not react with guaiacol and lack staining reactions on gallic and tannic acid agars. Most species of *Lentinus* produce basidiomata which are centrally stipitate whereas the basidiomata of *Lentinellus* species can be centrally to laterally stipitate or sessile.

Lentinula Earle -- The genus *Lentinula* contains only two species, *L. boryana* (Berk. & Mont.) Pegler and *L. edodes* (Berk.) Pegler, which are distinguished from *Lentinellus* by smooth, ellipsoid-cylindric, inamyloid basidiospores, inflated generative hyphae, and a central to eccentric stipe (Pegler, 1975). *Lentinellus* has a monomitic hyphal system composed of thin- to thick-walled, non-inflated, generative hyphae.

Pleurotus (Fr.) Quel. -- *Pleurotus* features smooth, cylindric, inamyloid basidiospores (Largent and Baroni, 1988), even lamellar margins, and a dimitic hyphal system. *Lentinellus* has serrate to serrulate lamellar margins.

Panus Fr. -- *Panus* is distinguished from *Lentinellus* by smooth, cylindric, inamyloid basidiospores, even lamellar margins, and a dimitic hyphal system (Largent and Baroni, 1988).

Panellus Karst. -- The basidiospores of *Panellus* are smooth, cylindric, elongate-ellipsoid, oblong or allantoid (Largent and Baroni, 1988). In addition,

the lamellar margins are even and some taxa produce a gelatinous zone in the pileus trama. *Lentinellus* does not exhibit a gelatinous zone in the pileus trama.

Hohenbuehelia Schulz. -- *Hohenbuehelia* is recognized by smooth or finely punctate, inamyloid basidiospores, gelatinous zone in the pileus trama, and thick-walled metuloids in the hymenium (Largent and Baroni, 1988). Metuloids are not present in *Lentinellus*.

Resupinatus Nees: S.F. Gray -- The basidiocarps of *Resupinatus* are relatively small, grayish to fuscous, and produce a lateral stipe, pseudostipe or are sessile (Largent and Baroni, 1988). The basidiospores are inamyloid, globose, ellipsoid, allantoid or cylindric, and the hyphae of the pileus and lamellar tramas are gelatinized. The basidiocarps of *Lentinellus* are buff to reddish-brown or blackish-brown and the pileus and lamellar tramas are never gelatinized.

Although the genus *Lentinellus* is readily recognized by serrate lamellae and amyloid, ornamented basidiospores, several species, especially the non-stipitate taxa, are extremely variable and difficult to distinguish macroscopically and/or microscopically. The difficulty in segregating taxa is due primarily to the relatively long life of the basidiomata which results in considerable variation in the pileus tomentum and degree of tramal amyloidity (Miller and Stewart, 1971). In fact, the use of morphological characteristics to segregate taxa has led to considerable confusion as numerous taxa have been proposed only to later be reduced to synonymy (Miller and Stewart, 1971; Pegler, 1983; Segedin, 1996).

Among the most confusing species complex is the one which includes *L. ursinus*, *L. castoreus*, a European taxon, *L. vulpinus*, and several other morphospecies (Stalpers, 1996) which have not been adequately differentiated biologically or phylogenetically from *L. ursinus sensu stricto*. To date, neither mating studies nor molecular analyses have been employed to further distinguish these morphologically similar taxa.

Fries (1821) originally described the basidiomata of *L. ursinus* as having a yellow-brown, glabrous to tomentose pileus, lamellae 9-13.5 mm broad, and sessile, imbricate growth form. Although his (Fries, 1821) original description of *L. vulpinus* revealed little morphological data, Fries (1838, 1863) later described the basidiomata of this taxon as having a pale brown, squamulose to warty pileus with superficial, longitudinally raised ribs, and growth form in tightly imbricate clusters. Fries (1838) subsequently proposed *L. castoreus* with a brief description which he later expanded (Fries, 1863) to include basidiomata with a pale reddish-brown, ribbed to rugose, glabrous pileus, lamellae 2.5-5 mm broad, and growth form in subsessile, imbricate clusters.

Although Pilat (1946) later considered *L. castoreus* and *L. ursinus* to be synonymous with *L. vulpinus*, Romagnesi (1946) maintained that the three taxa were distinctive. Romagnesi (1946) described *L. castoreus* as having 4-12 cm broad, tan, finely tomentose pilei with a pulverulent margin, smooth, weakly amyloid basidiospores, strongly amyloid trama, and habitat on coniferous trees; *L. ursinus* as producing 2-4 cm broad, brown, velvety pilei with a glabrous

margin, verrucose, strongly amyloid basidiospores, moderately amyloid trama, and habitat on deciduous trees (especially *Fagus*); and, *L. vulpinus* with 3-15 cm broad, alutaceous, radially veined, glabrous pilei, very strongly amyloid basidiospores, and amyloid trama. Kotlaba and Pouzar (1965) later defined differences between *L. castoreus* and *L. ursinus* based on basidiomata size, degree of tramal amyloidity, and substrate preference. They (Kotlaba and Pouzar, 1965) described *L. castoreus* as having 3.5-15 cm broad pilei, strongly amyloid trama, and habitat on coniferous and deciduous trees while *L. ursinus* had 1-5 cm broad pilei, weakly amyloid trama, and habitat only on deciduous trees (especially *Fagus*, *Carpinus*, and *Quercus*). According to Kotlaba and Pouzar (1965), *L. castoreus* is a larger taxon than *L. ursinus*, produces broader lamellae (4-6 mm vs. 2-3 mm), and features a pileus tomentum which does not extend to the margin. Clearly, these workers (Romagnesi, 1946; Kotlaba and Pouzar, 1965) have a different concept of these taxa than Fries. Based on Fries' (1821, 1838, 1863) descriptions, one might assume that broader lamellae were produced by the taxon with larger basidiomata and that *L. ursinus* is the larger taxon not *L. castoreus*. One solution to the confusion regarding taxonomic concepts in this species complex is typification of *L. castoreus*, *L. ursinus*, and *L. vulpinus* so that the circumscriptions of these taxa can be stabilized and voucher specimens designated for macromorphological comparison.

Miller and Stewart (1971) considered *L. castoreus* to be a European species that is synonymous with *L. ursinus*. They segregated *L. ursinus* from *L.*

vulpinus based on macromorphological features which adhered to Fries' original descriptions (1821, 1838, 1863) as well as micromorphological features. *Lentinellus ursinus* possesses a pileipellis composed of parallel, radially arranged hyphae which give rise to pileocystidia that are solitary or in fascicles and has fusiform pseudocystidia. *Lentinellus vulpinus* exhibits a pileipellis of tangled hyphae which lacks pileocystidia, hypodermium composed of thick-walled hyphae, and lacks fusiform pseudocystidia.

D. Mating Studies

Although Vandendries first suggested the use of mating studies for determining fungal species in 1923, morphological analysis has continued to govern fungal taxonomy and mating studies have rarely been employed for defining species (Anderson and Ullrich, 1982; Vilgalys and Miller, 1987; Petersen and Bermudes, 1992; Petersen and Methven, 1994). Boidin (1986) described the usefulness of mating studies as follows: 1) to determine if morphologically similar species are reproductively isolated; 2) to define the limits of morphological variation within a single biological species; 3) to identify interincompatible sibling species; 4) to determine the geographical range of biological species; and, 5) to demonstrate gradual speciation in members of a single morphospecies.

Presently, two types of heterothallic mating systems have been identified in basidiomycetes: unifactorial or bipolar heterothallism and bifactorial or

tetrapolar heterothallism. In bipolar mating systems, reproductive compatibility is controlled by a single gene with multiple alleles designated A_1, A_2, A_3,...A_n. Since two alleles are present in an individual dikaryotic mycelium, only two mating types (e.g., A_1 and A_2, or A_1 and A_3, etc.) can be identified. In tetrapolar mating systems, reproductive compatibility is controlled by two genes each with multiple alleles designated A_1, A_2, A_3,...A_n and B_1, B_2, B_3,...B_n, respectively. While only two alleles are present in an individual dikaryotic mycelium, four mating types (A_1B_1, A_2B_2, A_1B_2, A_2B_1, or A_1B_1, A_3B_3, A_1B_3, A_3B_1, etc.) can be identified. Compatible matings (termed "positive") only occur between mycelia with different alleles (e.g. A_1 X A_2 or A_1B_1 X A_2B_2). The type of mating system present is determined by pairing monokaryotic isolates, derived from individual basidiospores from a single basidiocarp, in all possible combinations (termed a "self-cross"). Positive "matings" are indicated by the formation of clamp connections which develop after plasmogamy between compatible monokaryons and the establishment of a stable dikaryon. Positive "matings" result in approximately half the total crosses in bipolar mating systems and one quarter the total crosses in tetrapolar mating systems. After a mating system has been determined, tester strains representative of each mating type are arbitrarily assigned. Tester strains are then "mated" with other tester strains or undetermined single basidiospore isolates from the same or different taxa to determine intercompatibility between populations.

E. Culture Mat Analysis and Phenoloxidase Activity

Since sexually incompatible populations have been shown to possess unique morphological features which can be used to differentiate biological species (Boiden, 1986; Berube and Dessureault, 1987; Mueller, 1991; Vilgalys, 1991), culture mat analyses and phenoloxidase reactions can be employed to provide additional macroscopic and microscopic characteristics for separating putative biological species. In order to provide additional morphological characteristics for use in the systematics of wood-rotting Aphyllophorales, Nobles (1948, 1958a, 1965) proposed a method for the description of somatic cultures and provided keys for their identification. Stalpers (1978) advanced the study of the Aphyllophorales by including additional characteristics, including chemical spot tests, as a means for identifying 550 species in culture. The description of culture characteristics is now considered essential data in species descriptions in the Aphyllophorales. Miller (1971) indicated the potential value of culture characteristics in the taxonomy of the Agaricales, but to date, studies including descriptions of culture morphology have been limited (Vilgalys and Miller, 1983; Mueller and Fries, 1985; Thorn and Barron, 1986; Ovrebo, 1988; Desjardin, 1990; Johnson and Methven, 1994).

The type of wood rot produced by basidiomycetes is considered to be an important taxonomic characteristic and is determined through the analysis of phenoloxidase activity. Wood-rotting fungi can be classified into two large groups based on the type of rot they produce: 1) brown-rot fungi which degrade

cellulose and hemicellulose; and, 2) white-rot fungi which degrade cellulose, hemicellulose, and lignin. White-rot fungi are believed to contain the phenoloxidase enzymes laccase, tyrosinase, and peroxidase (Lyr, 1956, 1958a, 1958b) whereas brown-rot fungi lack these enzymes. Many tests for the detection of these enzymes have been developed. Bavendamm (1928) was the first to test for the presence of oxidative enzymes by growing fungi on malt agar containing tannic or gallic acid. White-rot fungi are readily identified by the production of a brown diffusion zone in the agar when oxidases are present whereas brown-rot fungi produce no change in the agar. Nobles (1958b), who searched for a more rapid test for differentiating white and brown-rot fungi, suggested applying guaiacol directly to the mycelium of actively growing cultures. The brown color of guaiacol is changed to blue only in the presence of phenoloxidases and provides a rapid test to identify white-rot fungi. Both tests were found to be inconsistent and neither can be used to differentiate between specific phenoloxidases (Kaarik, 1965). Boidin (1951) suggested adding 0.2% tyrosine or p-cresol to malt extract agar for the detection of tyrosinase and 0.2% guaiacol for the detection of laccase. He (Boidin, 1951) divided the wood-rotting fungi into four groups based on the specific enzymes present in the mycelium: 1) fungi possessing neither laccase or tyrosinase (i.e., brown-rot fungi); 2) fungi possessing only laccase; 3) fungi possessing only tyrosinase; and, 4) fungi possessing both laccase and tyrosinase. Although more precise, these tests, like the Bavendamm test, were slow to develop and can take weeks to produce

positive results. In order to find a more rapid means of identifying specific phenoloxidases, Kaarik (1965) introduced chemical spot tests to analyze the reactions of fungal enzymes to 28 phenolic compounds. She suggested the use of benzidine and a-naphthol, rather than guaiacol, for the detection of laccase and p-cresol and tyrosine for the detection of tyrosinase. Taylor (1974) later proposed the use of pyrogallol plus hydrogen peroxide for the detection of peroxidase. Stalpers (1978) incorporated the results from each of these enzyme tests into his keys of the wood-inhabiting Aphyllophorales. Syringaldazine was subsequently proposed by Harkin and Obst (1973) as a more effective reagent for the detection of laccase. Marr (1979) also used syringaldazine for the detection of laccase and L-tyrosine and p-cresol for the detection of tyrosinase. Marr et al. (1986) defined four phenoloxidase profiles similar to those of Boidin (1951) and discussed the taxonomic potential of laccase and tyrosinase in the Basidiomycetes. As in this study, other workers (Overbo, 1988; Desjardin, 1990; Petersen, 1992; Johnson and Methven, 1994) have generally employed the methods of Marr (1979) for the detection of phenoloxidase activity in culture mat analyses.

II. MATERIALS AND METHODS

Basidiomata were collected in six Midwestern states from August through November, 1995 and 1996. Macromorphological features were recorded using standard techniques (Largent, 1977) with colors terms taken from Kornerup and Wanscher (1967). Photographs were taken with a Canon Rebel XS 35 mm camera using ASA 64 or 100 film. Spore prints were collected on white paper. Basidiomata were dried and deposited in the herbarium at Eastern Illinois University (EIU). Additional specimens were obtained from herbaria at Edinburgh (E), the University of Tennessee (TENN), the University of Michigan (MICH), and Uppsala (UPS). Supplementary specimens with spore prints were also provided by Dr. Andrew S. Methven (ASM) and Dr. Thomas J. Volk (TJV).

Micromorphological analyses of specimens followed standard practices and terminology (Largent et al., 1977). Dried basidiomata were reconstituted in distilled water and 95% ethanol. Hand sections of the pileus, lamellae, and stipe (if present) were made using a double-edged razor blade. Sections were mounted in 10% NH_4OH, Melzer's Reagent, or sulfobenzaldehyde and observed under bright field optics with a Nikon research microscope. Photomicrographs of micromorphological characteristics were taken with a Canon AE-1 35 mm camera mounted on a Nikon Labophot-2 research microscope using ASA 200 film. A minimum of 10 basidia, 10 pseudocystidia, and 30 basidiospores were measured for each collection.

A. Monospore and Polyspore Isolates

Single basidiospore isolates (SBIs) were collected utilizing one of the following methods: 1) Small sections of fresh basidiomata were suspended from the lid of a 90 mm pyrex petri plate with petroleum jelly and basidiospores allowed to fall onto Malt Extract (1.25% Difco) Agar (2% Difco Bacto) (MEA) for 15-60 minutes; or, 2) Basidiospores from a spore print were affixed to a moistened, sterilized loop and streaked onto the surface of MEA in a 90 mm pyrex petri plate. Plates were then wrapped in Parafilm and incubated in the dark at 23°C. After 3-6 days basidiospore germination occurred and individual germlings were transferred to MEA in 90 mm pyrex petri plates using a sterile probe and a Wild dissecting microscope. The germlings were allowed to grow until the hyphal mass was approximately 1 cm in diam, then transferred to individual screw cap test tubes containing MEA and stored in the dark at 23°C. Polyspore isolates were obtained by transferring a 5 mm^2 block of MEA containing numerous germlings to MEA in screw cap test tubes which were then stored in the dark at 23°C. In the case of ANM 321 and RHP 8768, polyspore isolates were created by self-crossing the mating types identified in each collection. Additional sets of SBIs, along with accompanying polyspore isolates and voucher specimens, were obtained from Dr. R.H. Petersen (RHP) (TENN). Ancillary sets of SBIs and voucher specimens from Austria and Costa Rica were obtained from Dr. I. Krisai-Greilhuber and Dr. R.H. Petersen, respectively. All

SBIs and polyspore isolates (Table VII) were deposited in the University of Tennessee Fungus Culture Collection (TENN).

B. Self-crosses and Matings

Single basidiospore isolates were transferred to MEA in 60 mm plastic disposable petri plates and incubated in the dark at 23°C. After hyphal growth covered the plates, the hyphae were examined for the presence or absence of clamp connections. An absence of clamp connections was used to confirm monokaryotic growth. In order to determine the mating system operating in each collection, the monokaryotic SBIs from each collection were crossed in all possible combinations by removing 5 mm diam plugs and placing them approximately 5 mm apart on MEA in 60 mm plastic disposable petri plates. The crosses were allowed to grow in the dark at 23°C until a substantial interface zone had formed (usually 10-14 days). A small section of the interface zone was removed, placed in a 1:1 mixture of 2% phloxine and 2% congo red, and examined at 400X under bright field illumination using a Nikon research microscope. Matings were scored on a grid for the presence (+) or absence (-) of clamp connections and notes on contact zone morphology recorded. Macromorphological features were recorded using the same procedure outlined under culture mat analyses (see below). After matings were scored, the grid was rearranged to produce the most discernable pattern (Ginns, 1974), and mating types assigned for each collection. Self-crosses were conducted on 13

of 15 collections (only 3 SBIs each were harvested from ANM 473 and ASM 8109). Tester strains, identified with an asterisk (*), were selected for intercollection matings. All tester strains (or designated SBIs if mating types were not identified) were crossed as above in all possible combinations. After ample time for development of an interface zone, matings were examined for clamp connections and scored as above.

C. Culture Mat Analysis and Phenoloxidase Activity

Polyspore isolates were transferred to the center of MEA in 90 mm pyrex petri plates and incubated in the dark at 23°C. After 11-14 days (28 days for RHP 7966), 5 mm diam plugs were removed from the margin of the hyphal mat and transferred to 12 replicate plates each of MEA and Difco Potato Dextrose Agar (PDA). Growth rates and macromorphological and micromorphological features were recorded at two week intervals according to Nobles (1948, 1965), Stalpers (1978), and Desjardin (1990). Color terms were taken from Kornerup and Wanscher (1967). Photographs of culture mats and photomicrographs of significant morphological characteristics were taken as outlined above at weeks two and six. Nobles Species Codes (1965) and Staplers Species Codes (1978) were determined for each species.

To determine the presence or absence of the phenoloxidase enzymes laccase, tyrosinase, and peroxidase, spot tests were conducted on culture mats at weeks two and six. Syringaldazine (Harkin and Obst, 1973, Harkin et al.,

1974) and 1-naphthol (Kaarik, 1965) were used to test for laccase activity, L-tyrosine and p-cresol for tyrosinase (Marr, 1984, Marr et al., 1986), and pyrogallol plus hydrogen peroxide for peroxidase (Taylor 1974, Staplers 1978). All reagents were prepared according to Marr (1979) except for pyrogallol plus hydrogen peroxide which was prepared according to Taylor (1974). Distilled water and 95% ethanol were used as negative controls. Spot tests were conducted using a procedure similar to that of Desjardin (1990). Seven plugs (5 mm diam) were removed from within 1 cm of the inoculation plug, and seven plugs were removed from the margin of actively growing cultures from one MEA and one PDA plate (Petersen pers. comm). Plugs were placed in individual 60 mm glass petri plates and each was flooded with a different reagent or control (5 reagents and 2 controls). Reactions were recorded as negative, weakly positive, or positive at 5, 10, 15, 30, and 60 minutes and at 24 hours. A positive reaction was recognized by the following color changes: 1) syringaldazine = pale yellow to magenta; 2) 1-naphthol = greyish red to violet; 3) L-tyrosine = colorless to reddish brown; 4) p-cresol = colorless to brownish orange; and, 5) pyrogallol plus hydrogen peroxide = pale yellow to yellowish brown. If the appropriate color change occurred within 30 minutes for syringaldazine or pyrogallol plus hydrogen peroxide or within 3 hours for 1-naphthol, L-tyrosine or p-cresol, the test was recorded as positive. If a color change occurred within these time limits but did not reach the required intensity, the test was recorded as weakly positive.

After these time periods the test was recorded as negative. Color terms for the reactions were taken from Kornerup and Wanscher (1967).

III. RESULTS

A. Description of Taxa

Lentinellus ursinus (Fr.) Kuhn., *Le Botaniste* 17: 99, 1926.

≡ *Agaricus ursinus* Fr., *Syst. Mycol.* 1: 185, 1821.

≡ *Lentinus ursinus* (Fr.) Fr., *Syst. Orb. Veg.* 1: 78, 1825.

≡ *Hemicybe ursina* (Fr.) Karst., *Hattsv.* 32: 248, 1879.

≡ *Panellus ursinus* (Fr.) Murrill, *N. Amer. Flora* 9(4): 246, 1915.

= *Lentinellus castoreus* (Fr.) Konrad and Maublanc, *Ic. Sel. Fung.* 6: 383, 1936.

≡ *Lentinus castoreus* Fr., *Epicr.*: 395, 1838.

≡ *Hemicybe castorea* (Fr.) Karst., *Hattsv.* 32: 249, 1879.

= *Lentinus anastomosans* Rick., *Lilloa* 2: 310, 1938.

= *Lentinus hepatotrichus* Berk., *In* Hook., *Fl. Tasm.* 2: 249, 1860.

= *Lentinus hyracinus* Kalchbr., *Grevillea* 8: 153, 1880.

= *Lentinus novae-zelandiae* Berk., *In* Hook., *Fl. New. Zeal.* 2: 176, 1855.

= *Lentinellus pusio* Romag., *Bull. Soc. Mycol. Fr.* 81: 71-74, 1965.

Pileus (1.5-) 2-14 x 2-10 cm, convex, conchate or plane in profile, dimidate, flabelliform or spathulate from above; margin incurved to decurved or plane, wavy to irregularly lobed; surface moist to dry, smooth or occasionally rugulose to rivulose near margin, margin nearly glabrous, light orange (5A4) to brownish orange (5C5) with a yellowish white (3A2-4A2) bloom which is easily removed, at times minutely felty at margin, yellowish white (3A2-4A2) to greyish

orange (5B4) or brownish orange (5C5), occasionally reddish brown (8E8) in age, velutinous to hirsute toward point of attachment, brownish orange (5C4-5) to light brown (5D5) or brown (6E4-5), hirsute hairs light brown (6D4) to brown (6E8) or dark brown (6F8); context up to 3 mm thick at broadest point, firm, yellowish white (2A2), pale yellow (3A3), light yellow (4A4-5) or greyish orange (6B2-3), not staining on exposure. **Lamellae** radiating outward from point of attachment, subdistant to close, thin, up to 8 (-11) mm broad, not forked, margins serrulate to coarsely serrate, lamellulae in 2-4 tiers; initially yellowish white (2A2-4A2) to pale yellow (3A3-4A3) or orange-white (5A2), becoming light yellow (4A4-5) in age, brown (6E4-5) where bruised. **Stipe** absent. **Odor** pleasant, faintly fruity to fungoid. **Taste** strongly acrid, numbing tip of tongue for 5 minutes or more.

Basidiospores white in mass, 3-5 x (2-) 2.5-4 um (3.86 $\pm$ 0.42 x 2.96 $\pm$ 0.29, Q = 1.30 $\pm$ 0.12), subglobose to broadly ellipsoid; thin-walled, minutely echinulate, amyloid; contents hyaline, light yellow in NH_4OH, frequently containing a globose, centric to subcentric, light yellow, refractive oil droplet. **Basidia** (9-) 12-22 x 3-5.5 um, narrowly clavate to clavate, thin-walled, four-sterigmate; contents hyaline to light yellow in NH_4OH. **Pseudocystidia** common on lamellar faces and edges (2 types): 1) (11-) 12-32 (-50) x 3-6 (-8) um, aculeate to subulate (fusiform), apices occasionally acuminate to mucronate, thin-walled, contents hyaline to light yellow in NH_4OH, occasionally resinous, staining dark purple in sulfobenzaldehyde; 2) (10-) 12-34.5 (-60) x 2-6.3 (-7) um,

cylindrical to clavate, apices obtuse, otherwise similar to Type 1 except contents are frequently resinous.

Pileipellis a cutis of radial hyphae and erect pileocystidia; radial hyphae (1-) 1.7-8 um diam, thin- to slightly thick-walled (<1 um), clamped, inamyloid, contents hyaline to yellow-brown in NH_4OH; pileocystidia 18-950 (-1600) x 1.5-5 (-8) um, solitary, tangled or in fascicles, thin-walled to slightly thick-walled (<1 um), clamped, inamyloid, contents light yellow to yellow-brown in NH_4OH. **Pileus trama** composed of loosely interwoven, thin- and thick-walled hyphae 1.5-8 um diam; thin- to slightly thick-walled (<1 um) hyphae clamped, inamyloid, contents hyaline in NH_4OH; thick-walled (walls 0.7-3 um thick) hyphae infrequently clamped, walls amyloid, reaction occasionally occurring in spiral bands, contents light yellow in NH_4OH. **Lamellar trama** similar to pileus trama. **Subhymenium** composed of parallel hyphae 2-8 um diam, thin-walled, frequently clamped, inamyloid, contents hyaline to caramel brown in NH_4OH, readily distinguished from thick-walled amyloid hyphae in trama. **Oleiferous hyphae** abundant throughout pileipellis, at times terminating as solitary, cylindric to narrowly clavate, thin-walled pileocystidia; infrequent to common in trama; abundant in subhymenium, terminating as pseudocystidia; thin-walled, unclamped, unbranched, inamyloid, yellowish in NH_4OH, always with resinous contents, staining dark purple in sulfobenzaldehyde.

Habitat: Solitary, caespitose or imbricate clusters on dead, decorticated, deciduous logs.

Distribution: Throughout temperate regions, common.

Commentary: *Lentinellus ursinus* is characterized macroscopically by the large (2-14 cm broad), brownish orange to yellow-brown pileus, subdistant to close lamellae, and sessile habit and microscopically by the subulate pseudocystidia.

Specimens utilized: RUSSIA: Primorski Region, District Ternei, Sichote Alin Biosphere Reserve, Kabanya, 18 Sep 1990, *R.H. Petersen* 3307 (TENN 53150); Primorski Region, District Ternei, Sichote Alin Biosphere Reserve, Meise, 8 Sep 1993, *R.H. Petersen* 6556 (TENN 52995). **SCOTLAND:** Hollands Wood, New Forest, Hants., 23 Oct 1969, *Orton* 3663 (E00028785) (E). **SWEDEN. Skane:** Osby, Kjelsved, 28 Sep 1980, *S. Ryman s.n.* (UPS); **Uppland:** Bondkyrka Par., Vardsatra Nature Reserve, 16 Oct 1980, *S. Ryman* 6132 (as *Lentinellus castoreus*) (UPS); Sollentuna Par., 400 m N. of Hagerstalund, 25 Sep 1984, *Nils Lundqvist* 15259 (F-01752) 18676 (UPS). **UNITED STATES. Illinois:** Jackson Co., Touch of Nature Preserve, 20 Oct 1989, *R.H. Petersen* 2414 (TENN 48582); Gallatin Co., Rim Rock Nature Preserve, 15 Oct 1994, *A.N. Miller* 210 (EIU); Douglas Co., Walnut Point State Park, 7 Oct 1995, *A.N. Miller* 321 (EIU); Ogle Co., White Pines Forest State Park, 28 Sep 1996, *A.N. Miller* 497 (EIU); Douglas Co., Walnut Point State Park, 4 Nov 1996, *A.N. Miller* 512 (EIU); **Indiana:** Brown Co., Yellowwood State Park, 20 Oct 1996, *A.N. Miller* 508 (EIU); Monroe Co., Hoosier National Forest, Col. Joshua Dettmer, 21 Oct 1996, *A.N. Miller* 510 (EIU); **Iowa:** Linn Co., Palisades-

Kepler State Park, 31 Aug 1996, *A.N. Miller* 480 (EIU); Muscatine Co., Wildcat Den State Park, Griss Mill Trail, 1 Sep 1996, *A.N. Miller* 482 (EIU); **Michigan:** Leelanau Co., North Bar Lake, 25 Jul 1995, *A.S. Methven* 8027 (EIU); Benzie Co., Esch Road, 7 Aug 1996, *A.S. Methven* 8109 (EIU); Leelanau Co., Shell Lake, 24 Jul 97, *A.S. Methven* 8155 (EIU); **Missouri:** Stoddard Co., Mingo Wildlife Refuge, 17 Sep 1994, *A.N. Miller* 169 (EIU); Wayne Co., Sam A. Baker State Park, Shut-in Trail, 20 Sep 1996, *A.N. Miller* 491 (EIU); Wayne Co., Mingo Wildlife Refuge, Ditch 6, 21 Sep 1996, *A.N. Miller* 493 (EIU); **North Carolina:** Transylvania Co., Pisgah National Forest, Coon Tree Trail, 29 Aug 1989, *R.H. Petersen* 2210 (TENN 48125); **Wisconsin:** La Crosse Co., Hixon Forest, 21 Aug 1996, *T.J. Volk* 96-105 (*A.N. Miller* 473, EIU).

Lentinellus angustifolius (Romell) Singer, *Lilloa* 25: 91, 1952.

≡ *Lentinus angustifolius* Romell, *Bih. K. svenska Vetensk Akad. Handl.* 26, Afd. 3, no. 16: 7-8, pl. 1/11, 1901.

Pileus 1-2.5 (-5) x 1-3.5 cm, conchate to plane in profile, dimidate to spathulate from above; margin decurved to plane, even to lobed, at times rimose; surface moist to dry, hygrophanous, margin glabrous, yellowish white (4A2), light yellow (4A4), greyish yellow (4B4), greyish orange (5B6) or brownish orange (5C6), minutely tomentose to matted fibrillose towards point of attachment, hispid hairs sometimes present, yellowish brown (5D6-5F6), brown (6E8) or dark brown (6F7); context thin, 1-1.5 mm thick, yellowish white (2A2) to

pale yellow (3A3), not staining on exposure. **Lamellae** radiating outward from point of attachment, close to crowded, thin, 2-3 mm broad, not forked, margins serrulate to serrate, lamellulae in 3-4 tiers; yellowish white (3A2) to pale yellow (3A3-4A3). **Stipe** absent. **Odor** pleasant, faintly fruity. **Taste** absent to slightly acrid, numbing tip of tongue for 5 minutes or more.

Basidiospores white in mass, (3.1-) 3.9-5 x (2.5-) 2.9-3.9 um (4.06 $\pm$ 0.29 x 3.10 $\pm$ 0.24, Q = 1.31 $\pm$ 0.09), subglobose to broadly ellipsoid; thin-walled, minutely echinulate, amyloid; contents hyaline, light yellow in NH_4OH, often containing a globose, centric to subcentric, light yellow, refractive oil droplet. **Basidia** (9-) 12-21 x 3-5.5 um, narrowly clavate to clavate, thin-walled, four-sterigmate; contents hyaline to light yellow in NH_4OH. **Pseudocystidia** common on lamellar faces and edges (2 types): 1) 13-29 (-39) x 3-6.5 um, subulate (fusiform) to obclavate, apices occasionally acuminate to mucronate, thin-walled, contents hyaline to light yellow in NH_4OH, occasionally resinous, staining dark purple in sulfobenzaldehyde; 2) 13-33 x 2-6 (-9) um, cylindrical to clavate, apices obtuse, otherwise similar to Type 1 except contents are frequently resinous.

Pileipellis a cutis of radial hyphae and erect pileocystidia; radial hyphae 1.7-8.5 um diam, thin- to slightly thick-walled (<1 um), clamped, inamyloid, contents light yellow to yellow-brown in NH_4OH; pileocystidia 30-900 (-1850) x 1.7-5 (-8) um, solitary, tangled or frequently in fascicles, thin-walled to slightly thick-walled (<1 um), clamped, inamyloid, contents light yellow to yellow-brown in NH_4OH. **Pileus trama** composed of interwoven, thin- and thick-walled hyphae

1.9-7 um diam; thin- to slightly thick-walled (<1 um) hyphae clamped, inamyloid, contents hyaline in NH_4OH; thick-walled (walls 0.5-3 um thick) hyphae rarely clamped, walls weakly amyloid, reaction occasionally occurring in spiral bands, contents light yellow in NH_4OH. **Lamellar trama** similar to pileus trama. **Subhymenium** composed of parallel hyphae 1.7-6.5 um diam, thin-walled, frequently clamped, inamyloid, contents light yellow to light caramel brown in NH_4OH. **Oleiferous hyphae** abundant throughout pileipellis, at times terminating as solitary, cylindrical, thin-walled pileocystidia; common in trama; abundant in subhymenium, terminating as pseudocystidia; thin-walled, unclamped, unbranched, inamyloid, yellow in NH_4OH, always with resinous contents, staining dark purple in sulfobenzaldehyde.

Habitat: Solitary to gregarious or imbricate clusters on dead, decorticated, deciduous logs.

Distribution: Throughout temperate regions in the United States, Illinois south to Mississippi and North Carolina west to Missouri, infrequent. Reported from Florida by Singer (Singer and Digilio, 1952). Also known from Costa Rica and Austria.

Commentary: *Lentinellus angustifolius* is characterized macroscopically by the small (1-2.5 cm broad), yellow-brown pileus, close to crowded, narrow lamellae, and sessile habit and microscopically by the subulate pseudocystidia.

Romell (1901) originally described this species as *Lentinus angustifolius* from Brazil based on two collections made by Malme in 1893. Although Romell

acknowledged the close affinity of *L. angustifolius* with *L. ursinus*, he referred to Fries' description of *L. ursinus* as having "lamellae 4-6 lin. wide." Singer and Digilio (1952) later transferred this taxon to *Lentinellus*, described it in more detail, and admitted that, "it was impossible to say if this is or is not a good species different from *Lentinellus ursinus*." Similar material was reported by Singer from Florida and by Wright from Cuba (as *Lentinus castoreus* var. *pusillus* Berk. & Curt.; Singer and Digilio, 1952). Based on mating studies and morphological features of the basidiomata and somatic cultures, *L. angustifolius* appears to be a species which can be adequately differentiated from *L. ursinus*.

The present author is using *L. angustifolius* as the name of the morphospecies with the above characteristics. This may not be the correct name since older names such as *Lentinus novae-zelandiae* Berk., which was placed in synonomy with *L. ursinus* (Pegler, 1983; Segedin, 1996), and *L. castoreus*, which also was synonomized with *L. ursinus* (Miller and Stewart, 1971; Pegler, 1983), closely resemble *L. angustifolius*. After describing *L. angustifolius* as a new species, Romell (1901) pointed out that he had not seen *L. novae-zelandiae*. Now that the *L. angustifolius* morphospecies has been found to be a distinct biological species, material representing *L. novae-zelandiae* and *L. castoreus* must be examined. If synonymy exists among these morphospecies, then the oldest name must be typified and the nomenclature reorganized in order to stabilize the use of these names.

Specimens utilized: AUSTRIA: Federal State, Burgenland, Jennersdorf District, Minihof-Liebau Community, forest along M|hlgraben Street, grid square 9162/1, 21 Sep 1996, *I. Krisai-Greilhuber s.n.* (*A.N. Miller* 511, EIU). **COSTA RICA:** Providence San Jose, Jardin de Dota, GPS N 9° 42'52" W 83° 58'28", 15 Jun 1995, *R.H. Petersen* 7808 (TENN 53758); Providence San Jose, Valle Rio Sevegre, San Gerasdo, GPS N 9° 33'2" W 83° 48'27", 21 Jun 1995, *R.H. Petersen* 7880 (TENN 53831); Providence San Jose, Valle Rio Sevegre, San Gerasdo, GPS N 9° 33'2" W 83° 48'27", 21 Jun 1995, *R.H. Petersen* 7876 (TENN 53748); **UNITED STATES. Illinois:** Ogle Co., Castle Rock State Park, Wildlife Viewing Trial, 27 Sep 1996, *A.N. Miller* 495 (EIU). **Mississippi:** Pearl River Co., Henleyfield, GPS N 30° 40'010" W 89° 47'74", 1 Dec 1995, *T.J. Volk* 95-96 (EIU). **Missouri:** Wayne Co., Mingo National Wildlife Refuge, Flat Banks, 21 Sep 1996, *A.N. Miller* 492 (EIU). **North Carolina:** Macon Co., Blue Valley, Pickelsimer's Falls, Forest Service Road 79, 4 Aug 1996, *R.H. Petersen* 8768 (TENN 55196, *A.N. Miller* 377, EIU). **Tennessee:** Sevier Co., Great Smoky Mountains National Park, Rainbow Falls Parking Lot, 28 Jul 1989, *R.H. Petersen* 2036 (TENN 48636).

Lentinellus vulpinus (Sow.: Fr.) Kuhner & Maire, *Bull. Soc. Mycol. Fr.* 49: 16, 1934.

≡ *Agaricus vulpinus* Sow., *Engl. Fung.* 3: pl. 361, 1803.

≡ *Agaricus vulpinus* (Sow.) Fr., *Syst. Mycol.* 1: 273, 1821.

≡ *Lentinus vulpinus* (Sow.: Fr.) Fr., *Syn. gen. Lent.*: 12, 1836.

≡ *Hemicybe vulpina* (Sow.: Fr.) Karst., *Hattsv.* 32: 249, 1879.

≡ *Panellus vulpinus* (Sow.: Fr.) Murrill, *N. Amer. Flora* 9(4): 246, 1915.

= *Lentinus auricula* Fr., *Vet. Ak. Forhandl.*: 29, 1861.

= *Lentinus hygrophanus* Harz, *Bot. Zbl.* 37: 378, 1889.

= *Lentinus tomentellus* Karst., *Medd. Soc. Fauna Fl. Fenn.* 14: 79, 1887.

≡ *Hemicybe tomentella* (Karst.) Karst., *Finl. Basid.*: 140, 1889.

Pileus 5-10 (-25) cm broad, convex to conchate in profile; margin inrolled; surface obscurely ridged near margin, ridges at times raised and prominent in age, occasionally with raised or recurved squamules near margin, elsewhere smooth and evenly covered by a cottony to wooly pubescence forming a turf of short hairs, white to "pale pinkish buff" or "pale pinkish cinnamon." **Lamellae** short decurrent, thin ridges extending down stipe, close to crowded, broad, equal, often breaking transversely, margins roughly serrate, initially whitish, becoming "pale pinkish cinnamon," staining brown. **Stipe** short, stout, often fused to form a large common base; surface dry, pubescent, light brown. **Pileus and stipe context** solid, watery pliant to soft at the center, dingy white with a pinkish or light brownish hue, "pale pinkish cinnamon" to light "sayal brown," darkening near the lamellae. **Odor** not distinctive. **Taste** acrid.

Basidiospores white in mass, 3-5 x (2.3-) 2.5-4.1 um (3.97 $\pm$ 0.38 x 3.12 $\pm$ 0.31, Q = 1.27 $\pm$ 0.09), subglobose to broadly ellipsoid; thin-walled, minutely echinulate, amyloid; contents hyaline, appearing light yellow in NH_4OH,

frequently containing a globose, centric to subcentric, light yellow, refractive oil droplet. **Basidia** (11-) 12-21.5 x 3.5-6 um, narrowly clavate to clavate, thin-walled, four-sterigmate; contents hyaline to light yellow in NH_4OH. **Pseudocystidia** 13-32 x 3-6 (-6.5) um, clavate, thin-walled, contents light yellow to yellow in NH_4OH, frequently with resinous contents.

Pileipellis a cutis of radial hyphae and erect pileocystidia; radial hyphae 2-6 (-9) um diam, thin- to slightly thick-walled (<1 um), clamped, inamyloid, contents yellow to caramel brown in NH_4OH; pileocystidia 35-450 x 1.5-5 (-7) um, thin-walled to slightly thick-walled (<1 um), solitary, tangled or in fascicles, clamped, inamyloid, contents hyaline to light yellow in NH_4OH. **Subpellis** (rarely present) a parallel layer of thin- and thick-walled hyphae 2-7.5 um diam; thin-walled hyphae, inamyloid, contents hyaline in NH_4OH; thick-walled (walls 0.9-3.5 um thick) hyphae, walls amyloid, contents light yellow in NH_4OH. **Pileus trama** composed of loosely interwoven, thin- and thick-walled hyphae 2.5-8 (-12) um diam; thin- to slightly thick-walled (<1 um) hyphae clamped, inamyloid, contents hyaline in NH_4OH; thick-walled (walls 1-4 um thick) hyphae clamped, walls amyloid, contents light yellow in NH_4OH. **Lamellar trama** similar to pileus trama except hyphae interwoven to parallel. **Subhymenium** composed of parallel hyphae 2.3-8 um diam, thin-walled, frequently clamped, inamyloid, contents hyaline to caramel brown in NH_4OH, readily distinguished from thick-walled amyloid hyphae in trama. **Stipe trama** composed of tightly interwoven, inamyloid, thin-walled hyphae and amyloid, thick-walled hyphae; otherwise

similar to pileus trama. **Oleiferous hyphae** abundant throughout pileipellis, at times terminating as solitary, cylindrical to narrowly clavate, thin-walled pileocystidia; infrequent in trama; abundant in subhymenium, terminating as pseudocystidia; thin-walled, unclamped, rarely branched, yellowish in NH_4OH, always with resinous contents, staining dark purple in sulfobenzaldehyde.

Habitat: Imbricate clusters on deciduous trees, especially on living *Ulmus* spp., also reported on fallen logs of *Betula*, *Fagus* and *Populus*.

Distribution: Europe and North America, rare.

Commentary: *Lentinellus vulpinus* is characterized macroscopically by the large (5-10 cm broad), white to yellowish pileus, close to crowded lamellae, and short, fused stipes and microscopically by the lack of subulate pseudocystidia. The macroscopic description was adopted from Miller and Stewart (1971). Colors in quotation marks are from Ridgeway (1912).

Specimens utilized: SCOTLAND: Surrey, East Horsley, Mountain Wood 2, 18 Oct 1969, *Orton* 3662 (E00028786) (E). **SWEDEN. Uppland:** Uppsala, Kyrkogardsallen, 25 Aug 1945, *Seth Lundell s.n.* (UPS); Uppsala Par., Skarpan at Ekeby, 13 Sep 1981, *Nils Lundqvist* 13557 (UPS); Uppsala, Stadsskogen at Skoghall, 1 Sep 1983, *S. Ryman* 7246 (UPS). **UNITED STATES. Michigan:** Luce Co., Pine Stump Junction, 10 Aug 1959, *A.H. Smith* 61259 (MICH); Cheboygan Co., Colonial Point Hardwoods, 26 Aug 1960, *A.H. Smith* 63033 (MICH); Oakland Co., Proud Lake, 3 Sep 1966, *A.H. Smith* 73305

(MICH). **Minnesota:** Beltrami Co., Webster Lake, 25 Aug 95, *R.H. Petersen* 7966 (TENN 54380).

B. Mating Studies

All self-crosses revealed a tetrapolar mating system and at least three mating types were identified for each collection (Figs. 1-16). Two collections (ANM 492 and ANM 495) initially appeared to have bipolar mating systems as only two mating types were identified from each collection (Figs. 10 and 12). After additional SBIs were crossed with assigned mating types, three mating types were identified for each collection indicating the presence of a tetrapolar mating system (Figs. 11 and 13).

Tester strains (identified with an *) of each collection were crossed in all possible combinations except in ANM 511 and RHP 7880 (Table VIII; Figs. 17 - 23). Although crosses of most collections were either completely compatible or completely incompatible, a few were partially compatible (Table VIII; Figs. 17-20, 23). These crosses resulted in the delineation of three intersterility groups (e.g., biological species) which were identified as *L. ursinus*, *L. angustifolius*, and *L. vulpinus* based on morphological characteristics of basidiomata and somatic cultures.

C. Culture Mat Analysis and Phenoloxidase Activity

Lentinellus ursinus (n = 11)

Macromorphology: MEA: Radius and (means): Week II 37-65 mm (52.9 mm), plates covered by week IV; mat initially silky, translucent, becoming farinaceous, white (1A1) to light yellow (4A4-5) by Week VI; advancing zone appressed, silky; margin occasionally plumose, not distinct, translucent; plug initially undifferentiated, translucent, becoming farinaceous, white (1A1) to light yellow (3A4-5) by Week VI; reverse unchanged; odor none to faintly fruity. **PDA:** Radius and (means): Week II 27-66 mm (44.0 mm), Week IV 64-84 mm (71.5 mm) or plates covered, plates covered by week VI; mat subfelty, white (1A1) to light yellow (3A4), becoming farinaceous by Week VI; advancing zone appressed, plumose, narrow (2-5 mm), not distinct, translucent to yellowish white (1A2); plug farinaceous, white (1A1) to light yellow (3A4-4A5) by Week VI; reverse variable, unchanged to yellow-brown (5E4-7) or brown (6D7-6E6) under plug by Week VI; odor none to faintly fruity.

Micromorphology: MEA: Advancing zone hyphae 1.3-6.3 um diam, thin-walled, clamped, infrequently branched, rarely intertwined into mycelial cords, hyaline, contents light yellow in KOH, inamyloid; encrusted "balls" surrounding hyphae, 5-9 x 5.1-8 um, globose to subglobose, surface granular, hyaline to pale yellow in KOH; crystals absent. **Aerial hyphae** 1-6 um diam, thin-walled, clamped, infrequently branched, hyaline, contents light yellow in KOH, inamyloid; gloeocystidia common to abundant, cylindric (25-101 (-166) x

2-5 um), clavate (13-60 x 3-8 um) or ventricose-rostrate (15-68 x 3-8 (-10.5) um), terminal, basally clamped, thin-walled, rarely forked, yellow in KOH, contents resinous, partially refractive, staining dark purple in sulfobenzaldehyde; chlamydospores abundant, 4-9.5 (-15) x (-3) 4-6.3 (-7.7) um [mean = 7.41 $\pm$ 1.68 x 5.14 $\pm$ 1.46], subglobose to pyriform or broadly ellipsoid, mostly terminal, rarely intercalary, thick-walled (up to 1 um), hyaline to yellow in KOH, contents undistinctive to resinous; crystals few to abundant, grain-like to globular, rarely needle-like, refractive. **Submerged hyphae** 1-6 (-6.9) um diam, thin-walled, clamped, infrequently to regularly branched, hyaline, contents light yellow in KOH, inamyloid; crystals few to abundant, prismatic and slightly refractive or grain-like to globular and refractive. **PDA: Advancing zone hyphae** same as MEA except encrusted "balls" absent. **Aerial hyphae** same as MEA. **Submerged hyphae** same as MEA except crystals are cuboidal to quartz-like, infrequently globular or rarely needle-like, refractive.

Nobles species code: 2.3.15.34.36.38.39.44.50.54.60.

Stalpers species code: 1.2.3.6.13.14.15.18.20.24.30.31.36.38.39.45.52. 53.73.81.82.83.85.89.94.

Specimens utilized: ANM 321, ANM 473, ANM 482, ANM 491, ANM 497, ANM 508, ANM 512, ASM 8027, ASM 8109, RHP 2210, RHP 2414.

Phenoloxidase reactions for *L. ursinus* are shown in Tables I and II.

Lentinellus angustifolius (n = 4)

Macromorphology: MEA: Radius and (means): Week II 40-69 mm (57.3 mm), plates covered by week IV; mat initially silky, becoming subfelty

(in RHP 2036), never farinaceous, translucent, pale yellow (2A3-3A3) to light yellow (2A4-3A5); advancing zone appressed, silky, even, not distinct, translucent; plug undifferentiated, becoming silky, never farinaceous, translucent to pale yellow (2A3); reverse unchanged (brownish orange (6C6-8) to light brown (6D7) in RHP 8768); odor none to faintly fruity. **PDA:** Radius and (means): Week II 32-63 mm (46.8 mm), plates covered by week IV; mat subfelty, never farinaceous, translucent, white (1A1), pale yellow (2A3-4A3), yellow (3A6) or orange-yellow (4A6); advancing zone appressed, silky, occasionally plumose, narrow (1-7 mm), not distinct, translucent; plug subfelty to felty, never farinaceous, yellowish white (1A2-4A2) to yellow (3A6); reverse unchanged; odor none to faintly fruity. RHP 8768 (abnormal growth): PDA: Week II 20-35 mm (25.8 mm), Week IV 22-42 mm (29.9 mm), Week VI 25-53 mm (32.7 mm); mat subfelty, never farinaceous, white (1A1), greyish beige (4C2), yellowish brown (5D6) or brown (6E4); advancing zone appressed to submerged, undulating, not distinct, white (1A1) to brownish orange (5C4); plug subfelty, similar to mat; reverse pale yellow (3A3), brownish orange (5C6), brown (6E8) or dark brown (6F8), odor faintly fruity.

Micromorphology: MEA: Advancing zone hyphae 1.1-5.3 um diam, thin-walled, clamped, infrequently branched, hyaline, contents light yellow in KOH, inamyloid; crystals absent. **Aerial hyphae** 1-5.3 um diam, thin-walled, clamped, infrequently branched, hyaline, contents light yellow in KOH, inamyloid; hyphal swellings absent to common, subglobose, thin-walled to rarely thick-

walled, terminal or intercalary, hyaline to yellow in KOH, contents occasionally resinous; gloeocystidia common to abundant (absent in RHP 8768), frequently cylindric (23.5-135 x 2-4.5 um), infrequently ventricose-rostrate (25.5-68 x 4-8.9 (-10) um), rarely clavate, terminal, always basally clamped, thin-walled, rarely forked, yellow in KOH, contents resinous, partially refractive, staining dark purple in sulfobenzaldehyde; chlamydospores absent at Week II (except in RHP 8768), rare at Week IV, infrequent at Week VI, 6-11 x 4-7.5 um [mean = 8.36 $\pm$ 1.49 x 5.69 $\pm$ 1.01], subglobose to broadly ellipsoid, terminal or intercalary, thick-walled (up to 1 um), yellow in KOH, contents resinous; crystals abundant (absent in RHP 8768), grain-like, globular or needle-like, refractive. **Submerged hyphae** 1-6 um diam, thin-walled, clamped, infrequently to regularly branched, hyaline, contents light yellow in KOH, inamyloid; crystals few to common (absent in RHP 8768), prismatic or globular, refractive. **PDA: Advancing zone hyphae** same as MEA except hyphae sinuous in RHP 8768. **Aerial hyphae** same as MEA except hyphae sinuous and caramel brown in KOH (in RHP 8768). **Submerged hyphae** same as MEA except hyphae occasionally sinuous (in RHP 8768) and crystals globular, cubodial or quartz-like, refractive.

Nobles species code: 2.3.15.34.36.37.38.39.44.50.54.60.

Stalpers species code: 1.2.3.6.13.14.15.18.20.24.30.35.36.38.39.45.52.53.73.82.83.85.89.94.

Specimens utilized: ANM 492, ANM 495, RHP 2036, RHP 8768.

Phenoloxidase reactions for *L. angustifolius* are shown in Tables III and IV.

Lentinellus vulpinus (n = 1)

Macromorphology: MEA: Radius and (means): Week II 10-18 mm (13.4 mm), Week IV 34-46 mm (40.5 mm), Week VI 45-66 mm (58.6 mm); mat silky, never farinaceous, translucent; advancing zone appressed and submerged, silky and plumose, uneven, not distinct, translucent; plug undifferentiated to barely silky by Week VI, never farinaceous, translucent; reverse unchanged; odor none to faintly fruity. **PDA:** Radius and (means): Week II 15-22 mm (18.9 mm), Week IV 40-44 mm (41.9 mm), Week VI 53-66 mm (57.3 mm); mat silky, never farinaceous, translucent to white (1A1); advancing zone appressed, silky, even to slightly undulating, not distinct, translucent; plug undifferentiated to subfelty by Week VI, never farinaceous, translucent to yellowish white (1A2); reverse unchanged; odor none to faintly fruity.

Micromorphology: MEA: Advancing zone hyphae 1.5-5 um diam, thin-walled, clamped, infrequently to regularly branched, occasionally sinuous, hyaline, contents light yellow in KOH, inamyloid; encrusted "balls" surrounding hyphae, globose, surface granular, light yellow in KOH; crystals absent. **Aerial hyphae** 1.1-4 um diam, thin-walled, clamped, infrequently to regularly branched, hyaline, contents light yellow in KOH, inamyloid; gloeocystidia abundant, cylindric, cylindro-clavate or capitulate (60-136 x 2.5-6 um), terminal, basally clamped, thin-walled, yellow in KOH, contents resinous, partially refractive, weakly staining dark purple in sulfobenzaldehyde or unstaining; crystals absent.

Submerged hyphae 1.5-6 um diam, thin-walled, clamped, infrequently to regularly branched, hyaline, contents light yellow in KOH, inamyloid; encrusted "balls" surrounding hyphae, globose, surface granular, light yellow in KOH; crystals few to common, prismatic, slightly refractive. **PDA: Advancing zone hyphae** same as MEA except sinuous hyphae and encrusted "balls" absent. **Aerial hyphae** same as MEA except crystals common, globular, refractive. **Submerged hyphae** same as MEA except encrusted "balls" and crystals absent.

Nobles species code: 2.3.15.32.36.38.47.50.54.60.

Stalpers species code: 1.2.3.9.13.14.15.20.30.36.39.45.52.53.73.82.83. 89.94.

Specimen utilized: RHP 7966.

Phenoloxidase reactions for *L. vulpinus* are shown in Tables V and VI.

IV. DISCUSSION

A. Basidiome Morphology

Unique morphological features of the basidiomata can be used to differentiate species. *Lentinellus ursinus*, which is common in eastern North America, can be differentiated macroscopically by the large (2-14 x 2-10 cm), brownish orange to yellow-brown pileus, subdistant to close, broad lamellae, and sessile growth habit. It is microscopically distinguished by the amyloid trama and subulate pseudocystidia. *Lentinellus angustifolius*, which is infrequent in eastern North America, can be distinguished macroscopically by the small (1-2.5 x 1-3.5 cm), yellow-brown pileus, close to crowded, narrow lamellae, and sessile growth habit. It is microscopically identical to *L. ursinus* except for the weakly amyloid trama. *Lentinellus vulpinus*, which is rare in eastern North America, was only collected once during this study. It has a large (5-10 cm), white to yellowish pileus, close to crowded, broad lamellae, short, fused stipes, amyloid trama and infrequent clavate pseudocystidia.

The descriptions of *L. ursinus* and *L. vulpinus* presented are similar to those of Miller and Stewart (1971) except they described separate pleuro- and cheilocystidia. The term pseudocystidia (Singer, 1962; Largent et. al., 1977) was used in the present study to describe lamellar cystidia which are tramal in origin, project beyond the hymenium, and stain in sulfobenzaldehyde. Since the pseudocystidia are identical on the lamellar faces and edges, separate pleuro-

and cheilocystidia are not recognized. Illustrations of the two types of lamellar cystidia can be found in Miller and Stewart (1971).

B. Mating Studies

All collections which were self-crossed revealed a tetrapolar mating system. These results support the conclusions of Lamoure (1989) for *L. ursinus* and *L. vulpinus*. Lamoure (1989) also found a tetrapolar mating system in *L. castoreus* and *L. omphalodes*. In the present study, *L. angustifolius* was also found to possess a tetrapolar mating system.

Three biological species identified as *L. ursinus*, *L. angustifolius*, and *L. vulpinus* were found to occur in eastern North America. With some exceptions, intercollection matings of designated tester strains were completely compatible or completely incompatible. Although not patterned, partial compatibility usually occurred between collections from adjacent states suggesting that the same alleles may be operating in these populations. For example, the same two alleles were found in two collections of *L. ursinus* (ASM 8027 and ASM 8109) from Michigan. Since only three positive matings occurred in crosses of these collections (Fig. 18), mating types for the unknown single basidiospore isolates of ASM 8109 were based on the assigned mating types of ASM 8027. In addition, mating behavior in two pairs of isolates (ASM 8027-2 and ASM 8109-1, ASM 8027-13 and ASM 8109-3) was identical with all other tester strains (Figs. 18-19) suggesting the same mating types were present in each pair. Another

explanation for partial compatibility is that some matings produced sparse growth in which clamp connections were infrequent and may have simply been missed during scoring. The population biology of *L. ursinus* should be studied further to determine mating type allele segregation in eastern North America.

A single collection of *L. vulpinus* (RHP 7966) was completely incompatible with all collections of *L. ursinus* and *L. angustifolius*. Intercollection matings within *L. ursinus* (ANM 321, ANM 473, ANM 482, ANM 491, ANM 497, ANM 508, RHP 2210, RHP 2414, ASM 8027, ASM 8109) and *L. angustifolius* (ANM 492, ANM 495, RHP 2036, RHP 8768) were compatible but intercollection matings between each of these species were completely incompatible. Additional collections from Austria (ANM 511) and Costa Rica (RHP 7880) were compatible amongst themselves and with North American collections of *L. angustifolius* suggesting genetic barriers to gene flow do not exist between these allopatric populations. Ten collections of *L. ursinus* and six collections of *L. angustifolius* displayed intercollection compatibility suggesting multiple alleles (at least 18 and 12, respectively) must be operating in each species.

C. Culture Mat Analysis and Phenoloxidase Activity

Culture mat analyses provided additional morphological characteristics which could be used for separating these taxa. *Lentinellus ursinus* covered the surface of both MEA and PDA plates in four weeks, produced silky to subfelty, white to light yellow, farinaceous colonies, and developed abundant

chlamydospores and cylindrical, ventricose-rostrate or clavate gloeocystidia. *Lentinellus angustifolius* covered the surface of both MEA and PDA plates in four weeks, formed silky to subfelty, translucent to orange-yellow colonies which were never farinaceous, produced few chlamydospores, and developed abundant cylindrical, ventricose-rostrate or clavate gloeocystidia. *Lentinellus vulpinus* which did not cover the surface of either MEA or PDA plates after six weeks, formed silky, translucent to white colonies, produced no chlamydospores, and developed only cylindrical gloeocystidia.

Significant differences were not observed between the MEA and PDA grown isolates in each species except for the following: 1) the mats in *L. ursinus* were silky on MEA and subfelty on PDA; 2) the reverse surface in *L. ursinus* was unchanged on MEA and, in some isolates, brown on PDA; 3) the mats in *L. angustifolius* were frequently silky to rarely subfelty and translucent to light yellow on MEA and always subfelty and translucent to orange-yellow on PDA; 4) the submerged hyphae in *L.ursinus* and *L. angustifolius* produced prismatic crystals on MEA and cubodial to quartz-like crystals on PDA; and, 5) the culture mat in one isolate of *L. angustifolius* (RHP 8768) grew abnormally on PDA but not on MEA. Furthermore, RHP 8768 was significantly different microscopically from the other *L. angustifolius* isolates on both MEA and PDA in that it lacked gloeocystidia and crystals and produced chlamydospores at week II. *Lentinellus vulpinus* produced similar results on MEA and PDA.

All monokaryons used in the self-crosses were grown on MEA. The macromorphology of the monokaryons was identical to that found in the polyspores isolates except that some isolates of *L. vulpinus* produced clavarioid basidiomata. These were similar in appearance to those reported by Miller (1971) in *L. cochleatus*, *L. pilatii*, and *L. ursinus*. None of the *L. ursinus* isolates in this study produced basidiomata.

Miller (1971) briefly studied *L. cochleatus*, *L. pilatii*, and *L. ursinus* in culture and found cystidia and chlamydospores similar to those found in the present study in *L. ursinus* and *L. angustifolius*. Miller (1965) also described *L. montanus* in culture and found cystidia similar to those in *L. angustifolius*, *L. ursinus*, and *L. vulpinus*. As already stated by Miller (1971), gloeocystidia and/or chlamydospores appear to be unique characteristics of *Lentinellus* spp. in culture.

The results of the phenoloxidase reactions were similar for all three species (Tables I - VI). All three produced strong reactions for peroxidase and laccase, but only weak reactions for tyrosinase, even when L-tyrosine and/or p-cresol were placed directly on the surface of the cultures in MEA or PDA. These data suggest that peroxidase and laccase are produced in significant quantities, whereas tyrosinase, when present, is produced in lesser quantities. All three species are identified as white-rotters since they possess all three phenoloxidase enzymes.

The presence of laccase and tyrosinase in *L. ursinus* was also reported by Boidin (1951). Boidin, employing the methods previously described, found strong reactions for laccase using guaiacol but only weak reactions for tyrosinase using tyrosine. The presence of laccase in *L. ursinus* was also found by Piroard (1956). Employing the methods of Nobles (1948) and Boidin (1951), Piroard added gallic acid or guaiacol to malt agar to test for laccase and tyrosine or p-cresol to malt agar to test for tyrosinase. Although Piroard's (1956) tests determined the presence of laccase, they overlooked the small quantities of tyrosinase detected by Boidin (1951) and the present author. In this study, drop tests were used to detect the small quantities of tyrosinase present in the PDA plates.

The results between the MEA and PDA grown isolates were identical except tyrosinase was detected in the PDA plates (Tables I - VI). MEA grown isolates produced no traces of tyrosinase and further research is needed before any conclusions can be made on this data. Similar reactions were produced by the actively growing mycelium at the margin and the older mycelium 1 cm from the inoculation plug suggesting these enzymes are not localized in any particular area of the colonies. Tests conducted at weeks II and VI were similar in each species. L-tyrosine and p-cresol reacted similarly in the detection of tyrosinase whereas 1-naphthol was more variable than syringaldazine in the detection of laccase. Although 1-naphthol is laccase specific (Marr, 1979), it has been shown to produce variable results (Marr, 1979). In the present study, 1-naphthol

was also placed directly in the MEA and PDA plates as well as on the plugs. Strong reactions were usually produced in the plates even when weak reactions were found in the plugs suggesting the quantity of mycelium is a factor when using 1-naphthol. As such, 1-naphthol should be placed directly in the plates if it is to be used as an indicator of laccase.

V. SUMMARY

Three species identified as *L. ursinus*, *L. angustifolius* and *L. vulpinus* were found to occur in eastern North America. *Lentinellus angustifolius* was reported from five North American states as well as Austria and Costa Rica. Basidiome characteristics provide a morphological basis for distinguishing these biological species. The results of mating studies demonstrated that all three species exhibited tetrapolar mating systems and that these taxa are interincompatible. During this study the taxonomic value of culture mat analyses and phenoloxidase activity was assessed for these taxa. The growth rate of somatic cultures and production of gloeocystidia and/or chlamydospores are taxonomically important for separating these species. Evaluation of phenoloxidase activity showed *L. ursinus*, *L. angustifolius* and *L. vulpinus* to be white-rot fungi since they produced the three major phenoloxidases. Molecular analyses should be conducted in order to develop a phylogenetic concept for these species.

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Table I. Phenoloxidase reactions of *L. ursinus* culture mats at week II.

WEEK II	MEA		PDA	
Reagents	at margin	1 cm from inoculation plug	at margin	1 cm from inoculation plug
L-tyrosine	-	-	(- to weakly +)	(- to +)
p-cresol	-	-	(- to +)	(- to +)
pyrogallol + H_2O_2	+	+	+	+
1-naphthol	+	(weakly + to +)	(weakly + to +)	+
syringaldazine	+	+	+	+
distilled H_2O	-	-	-	-
95% ethanol	-	-	-	-

Table II. Phenoloxidase reactions of *L. ursinus* culture mats at week VI.

WEEK VI	MEA		PDA	
Reagents	at margin	1 cm from inoculation plug	at margin	1 cm from inoculation plug
L-tyrosine	-	-	(- to +)	(- to +)
p-cresol	-	-	(- to +)	(- to +)
pyrogallol + H_2O_2	+	+	+	+
1-naphthol	(- to +)	(- to +)	(- to +)	(- to +)
syringaldazine	+	+	+	+
distilled H_2O	-	-	-	-
95% ethanol	-	-	-	-

Table III. Phenoloxidase reactions of *L. angustifolius* culture mats at week II.

WEEK II	MEA		PDA	
Reagents	at margin	1 cm from inoculation plug	at margin	1 cm from inoculation plug
L-tyrosine	-	-	(- to +)	(- to +)
p-cresol	-	-	(- to +)	(- to +)
pyrogallol + H_2O_2	+	+	+	+
1-naphthol	(- to +)	(- to +)	(weakly + to +)	+
syringaldazine	+	+	+	+
distilled H_2O	-	-	-	-
95% ethanol	-	-	-	-

Table IV. Phenoloxidase reactions of *L. angustifolius* culture mats at week VI.

WEEK VI	MEA		PDA	
Reagents	at margin	1 cm from inoculation plug	at margin	1 cm from inoculation plug
L-tyrosine	-	-	(- to +)	(- to +)
p-cresol	-	-	(- to +)	(- to +)
pyrogallol + H_2O_2	+	+	+	+
1-naphthol	(- to +)	(- to weakly +)	(weakly + to +)	+
syringaldazine	(weakly + to +)	+	+	+
distilled H_2O	-	-	-	-
95% ethanol	-	-	-	-

Table V. Phenoloxidase reactions of *L. vulpinus* culture mats at week II.

WEEK II	MEA		PDA	
Reagents	at margin	1 cm from inoculation plug	at margin	1 cm from inoculation plug
L-tyrosine	-	Not enough	weakly +	weakly +
p-cresol	-	growth	-	weakly +
pyrogallol + H_2O_2	+	occurred for	+	+
1-naphthol	+	testing this	+	+
syringaldazine	+	area!	+	+
distilled H_2O	-	XXX	-	-
95% ethanol	-	XXX	-	-

Table VI. Phenoloxidase reactions of *L. vulpinus* culture mats at week VI.

WEEK VI	MEA		PDA	
Reagents	at margin	1 cm from inoculation plug	at margin	1 cm from inoculation plug
L-tyrosine	-	-	weakly +	-
p-cresol	-	-	weakly +	-
pyrogallol + H_2O_2	+	+	+	+
1-naphthol	weakly +	-	+	+
syringaldazine	+	+	+	+
distilled H_2O	-	-	-	-
95% ethanol	-	-	-	-

Table VII. Sources of single basidiospore isolates used in mating studies.

Biological species	Culture number	Geographic origin
Lentinellus ursinus	ANM 321	Illinois
	ANM 473	Wisconsin
	ANM 482	Iowa
	ANM 491	Missouri
	ANM 497	Illinois
	ANM 508	Indiana
	RHP 2210	North Carolina
	RHP 2414	Illinois
	ASM 8027	Michigan
	ASM 8109	Michigan
Lentinellus angustifolius	ANM 492	Missouri
	ANM 495	Illinois
	ANM 511	Austria
	RHP 2036	Tennessee
	RHP 7880	Costa Rica
	RHP 8768	North Carolina
Lentinellus vulpinus	RHP 7966	Minnesota

Table VIII. Intercompatibility relationships between monokaryotic tester strains representing three biological species within the *L. ursinus* complex from eastern North America, Austria (ANM 511), and Costa Rica (RHP 7880); + = completely compatible, - = completely incompatible, (+) = partially compatible.

	L. vulpinus	*L. ursinus*									*L. angustifolius*					
	7966	321	473	482	491	497	508	2210	2414	8027	8109	492	495	2036	8768	511	7880
7966	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
321	-		(+)	+	+	+	+	(+)	+	+	+	-	-	-	-		
473	-	(+)		+	(+)	+	+	+	+	+	+	-	-	-	-		
482	-	+	+		(+)	+	+	+	(+)	+	+	-	-	-	-		
491	-	+	(+)	(+)		+	+	+	+	+	+	-	-	-	-		
497	-	+	+	+	+		(+)	+	+	(+)	(+)	-	-	-	-		
508	-	+	+	+	+	(+)		+	+	(+)	(+)	-	-	-	-		
2210	-	(+)	+	+	+	+	+	+	+	+	+	-	-	-	-		
2414	-	+	+	(+)	+	+	+	+		(+)	(+)	-	-	-	-		
8027	-	+	+	+	+	(+)	(+)	+	(+)		(+)	-	-	-	-		
8109	-	+	+	+	+	(+)	(+)	+	(+)	(+)		-	-	-	-		
492	-	-	-	-	-	-	-	-	-	-	-		+	(+)	(+)	(+)	(+)
495	-	-	-	-	-	-	-	-	-	-	-	+		+	+	+	+
2036	-	-	-	-	-	-	-	-	-	-	-	(+)	+		+	(+)	(+)
8768	-	-	-	-	-	-	-	-	-	-	-	(+)	+	+		+	+
511												(+)	+	(+)	+		(+)
7880												(+)	+	(+)	+	(+)	

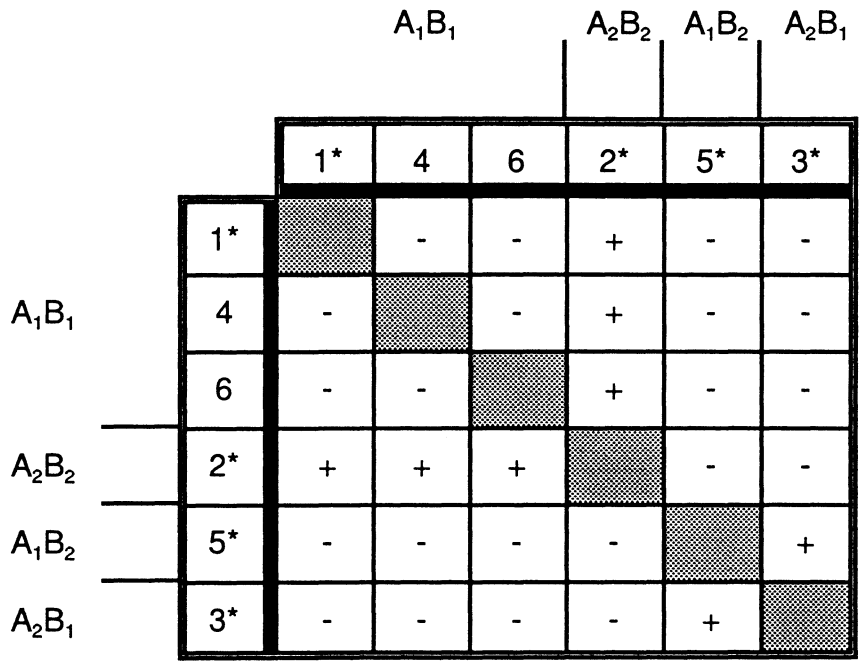


Fig. 1. Self-cross of *Lentinellus ursinus* ANM 321; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and assigned mating types at top and left.

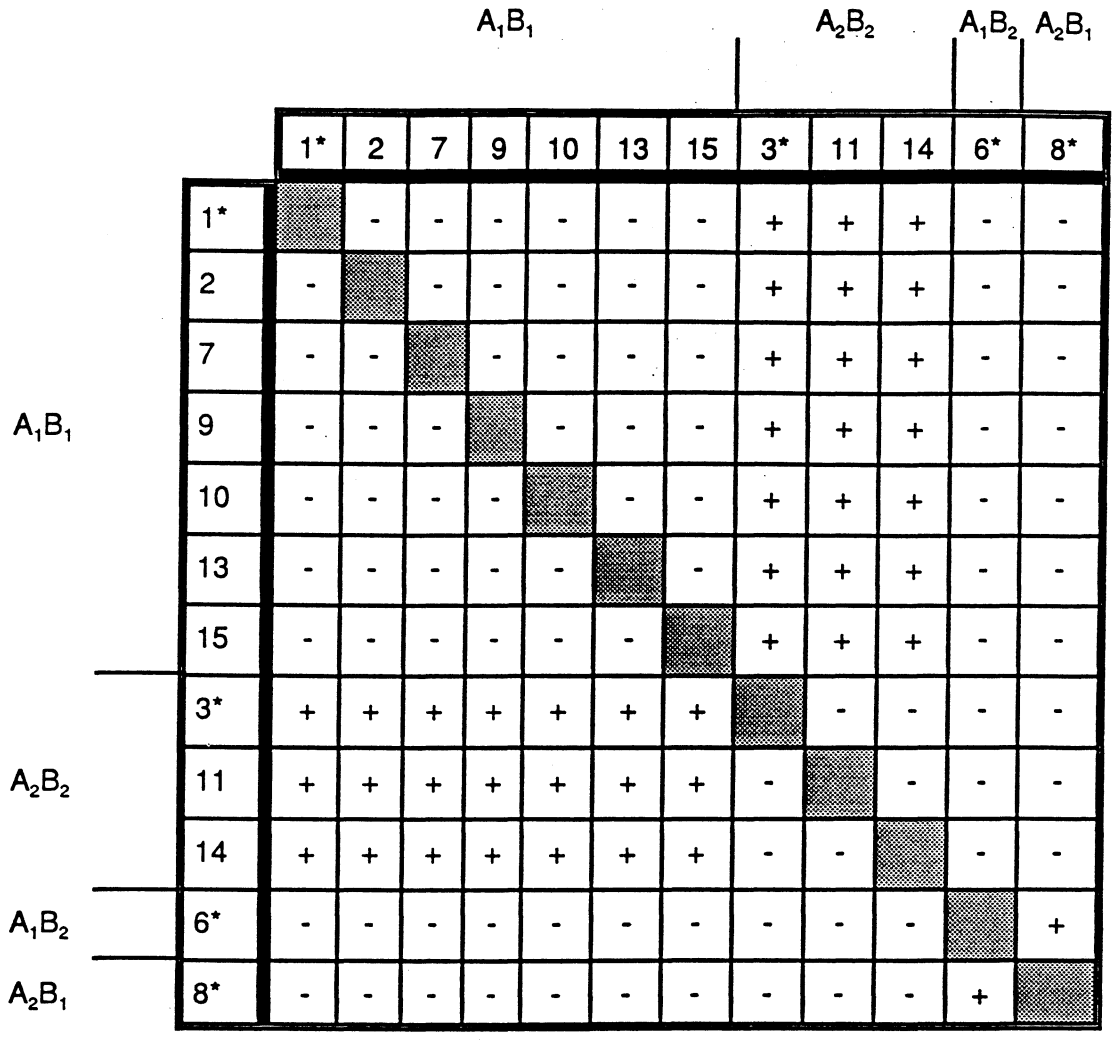


Fig. 2. Self-cross of *Lentinellus ursinus* ANM 482; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and assigned mating types at top and left.

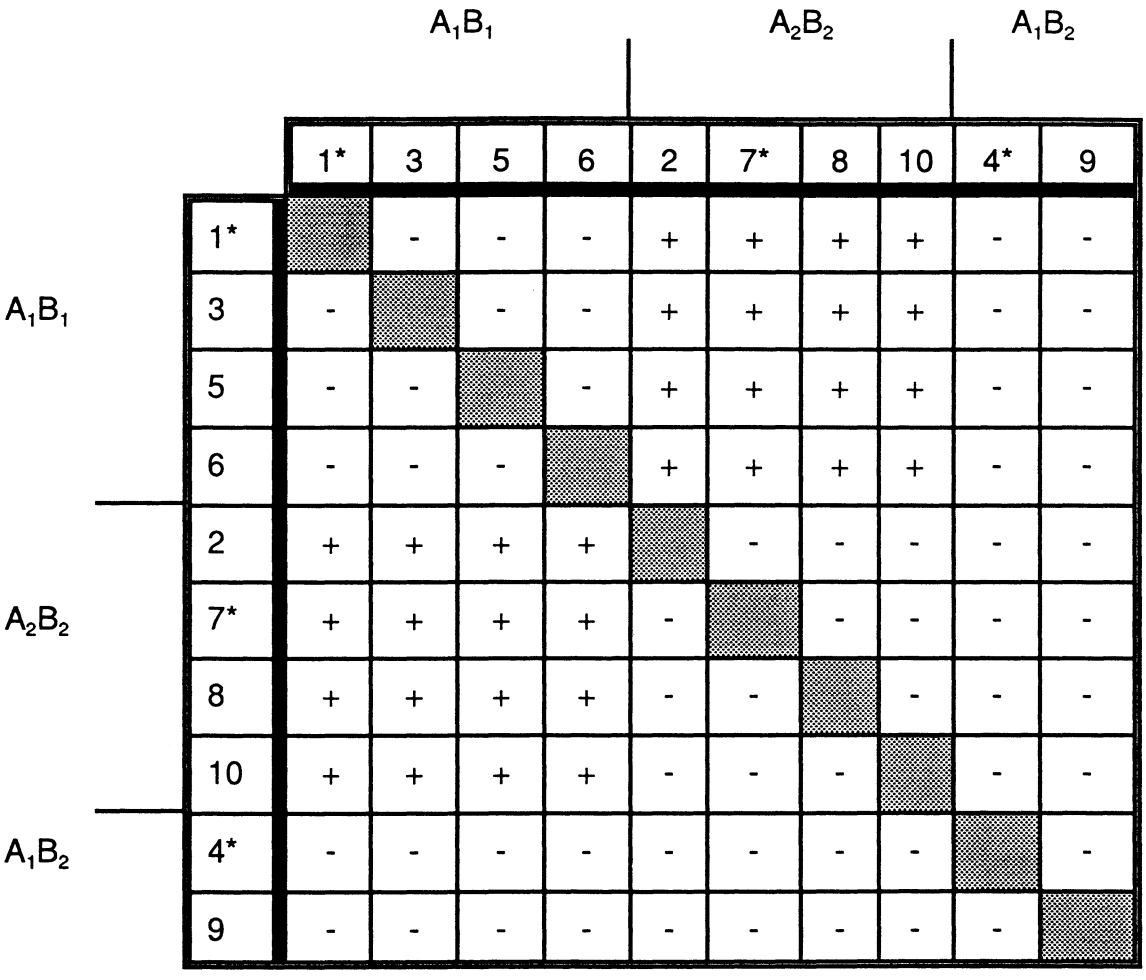


Fig. 3. Self-cross of *Lentinellus ursinus* ANM 491; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and assigned mating types at top and left.

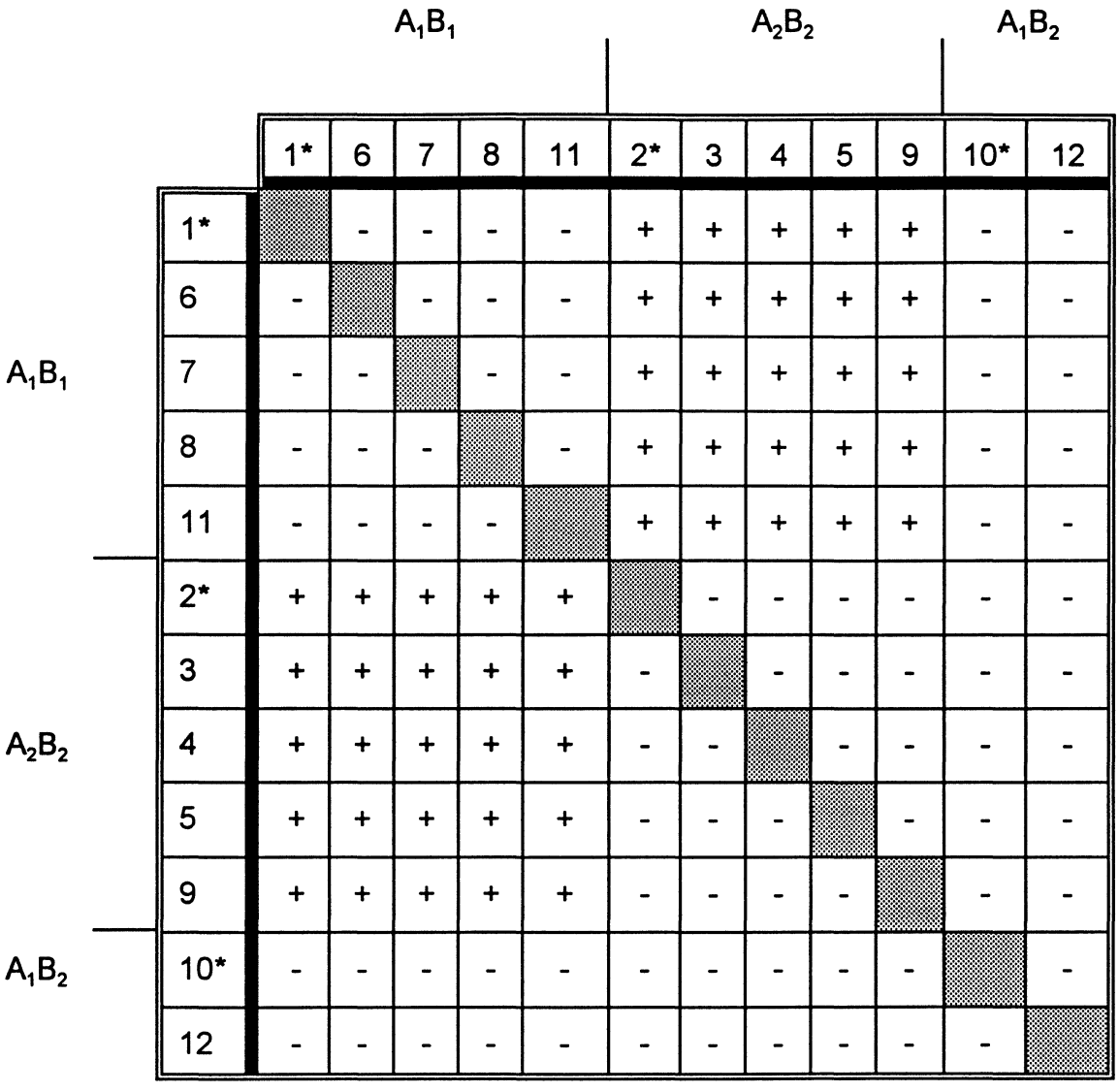


Fig. 4. Self-cross of *Lentinellus ursinus* ANM 497; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and assigned mating types at top and left.

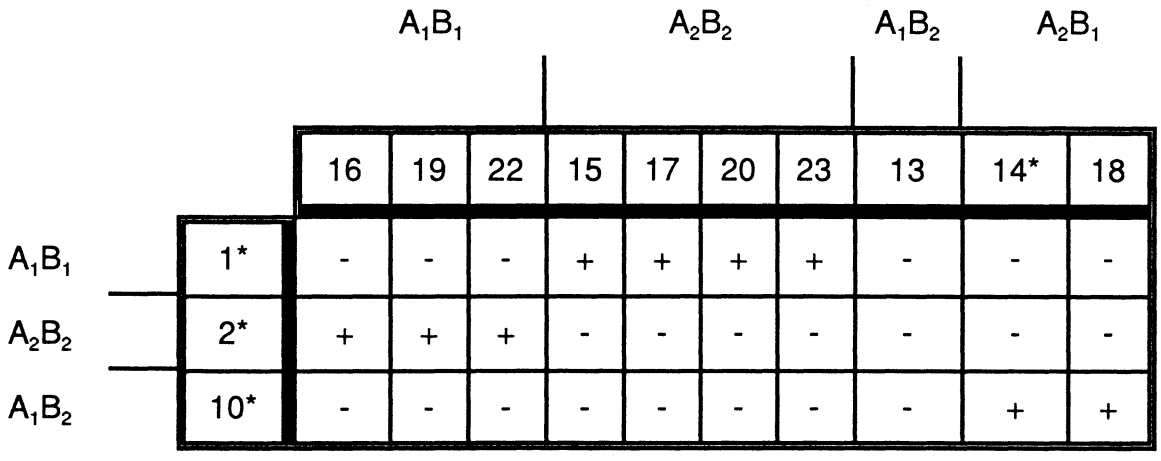


Fig. 5. Additional pairings of *Lentinellus ursinus* ANM 497 to identify A_2B_1 mating type; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and assigned mating types at top and left.

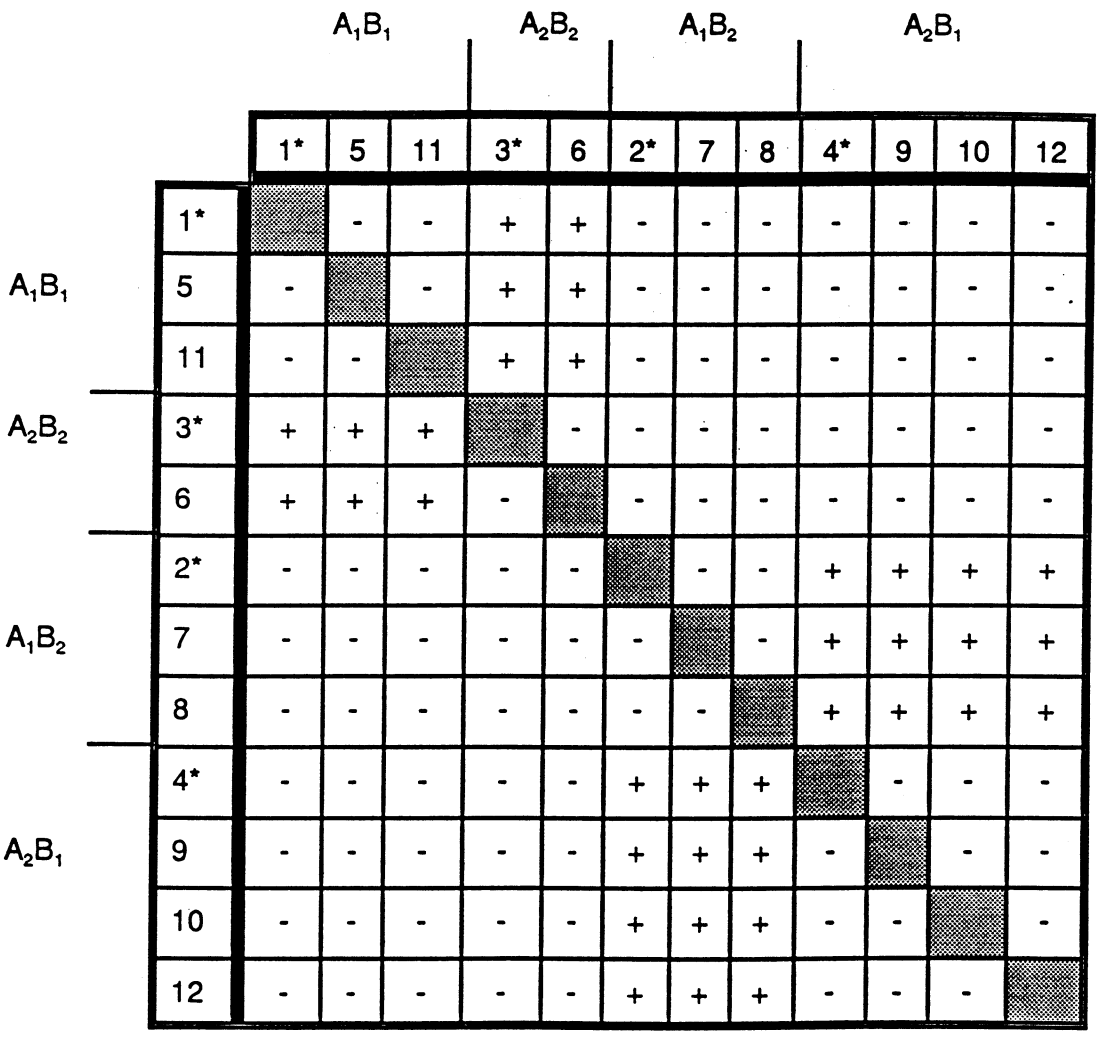


Fig. 6. Self-cross of *Lentinellus ursinus* ANM 508; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and assigned mating types at top and left.

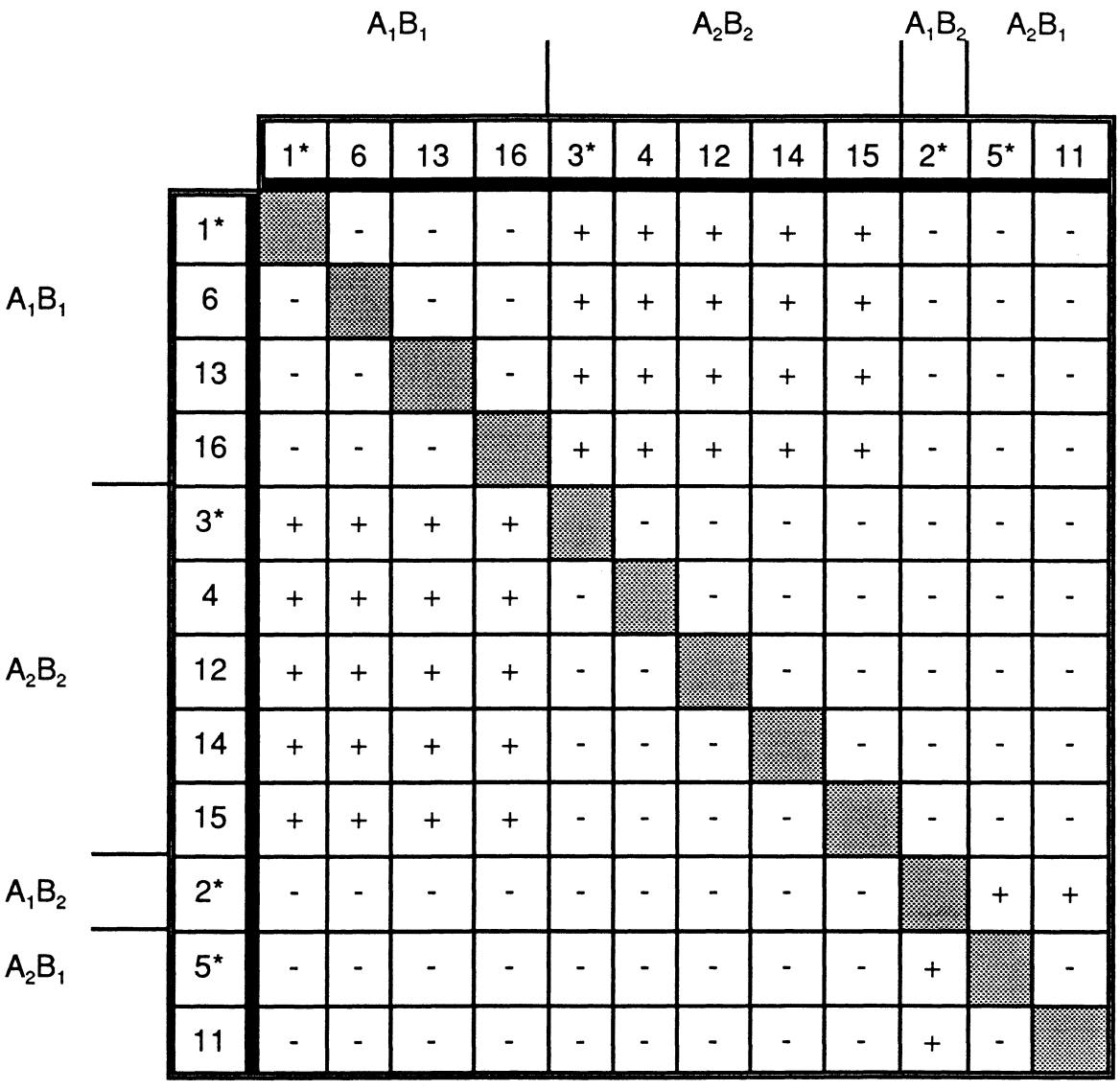


Fig. 7. Self-cross of *Lentinellus ursinus* RHP 2210; + = compatible mating, - = incompatible mating, * = tester strains. Isolate numbers and assigned mating types at top and left.

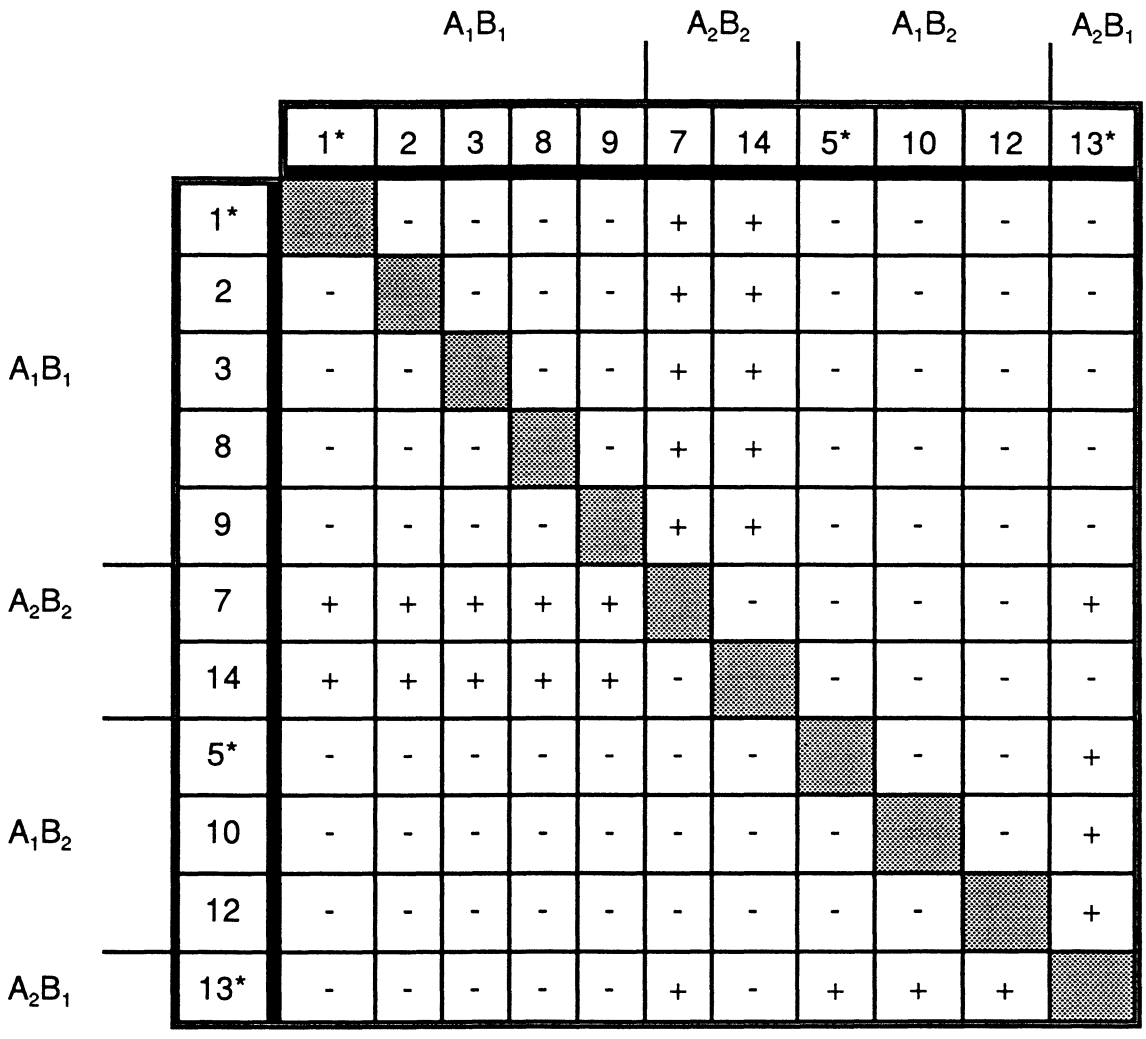


Fig. 8. Self-cross of *Lentinellus ursinus* RHP 2414; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and mating types at top and left. (Note: no tester strain was designated for the A_2B_2 mating type due to contamination.)

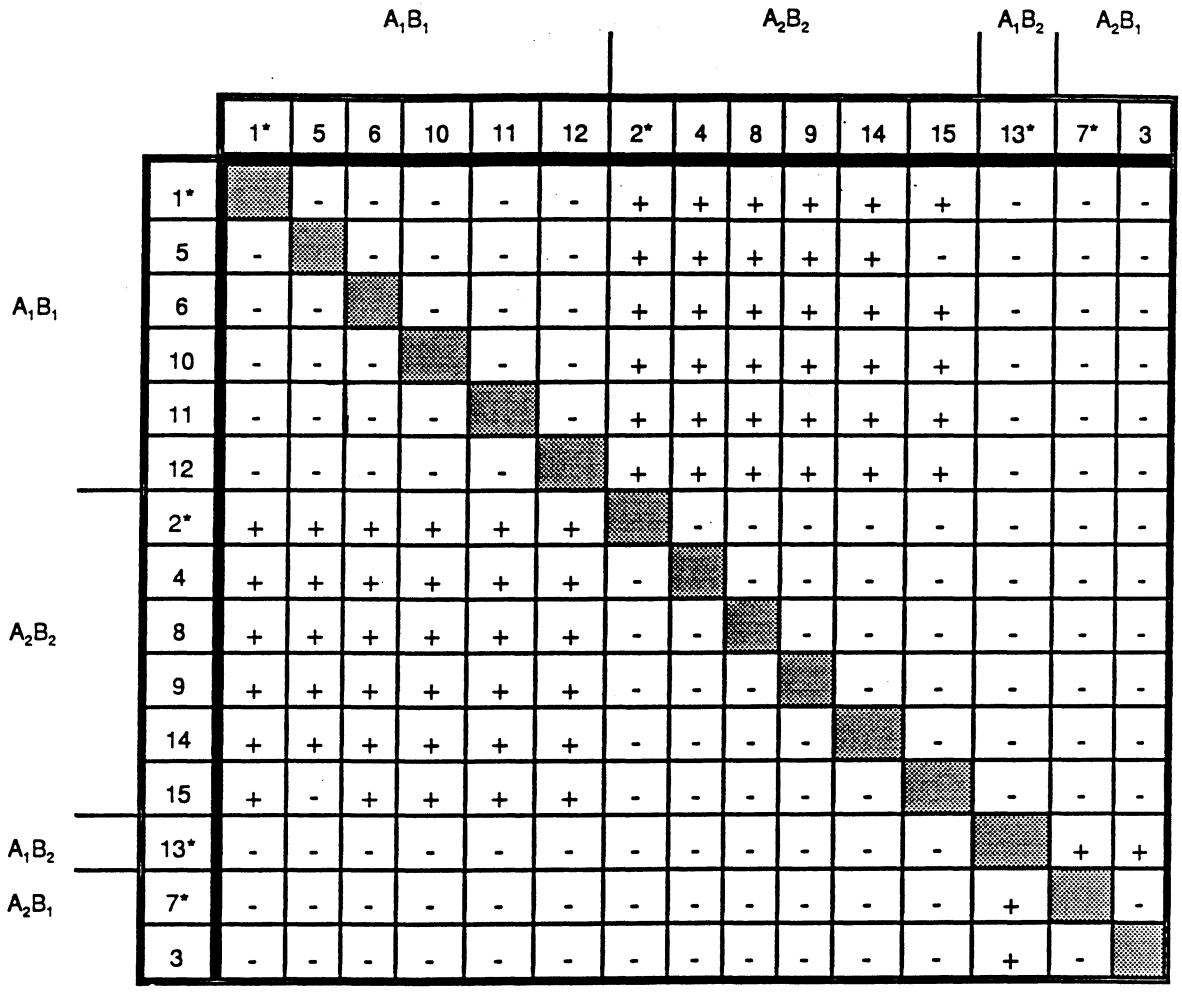


Fig. 9. Self-cross of *Lentinellus ursinus* ASM 8027; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and assigned mating types at top and left.

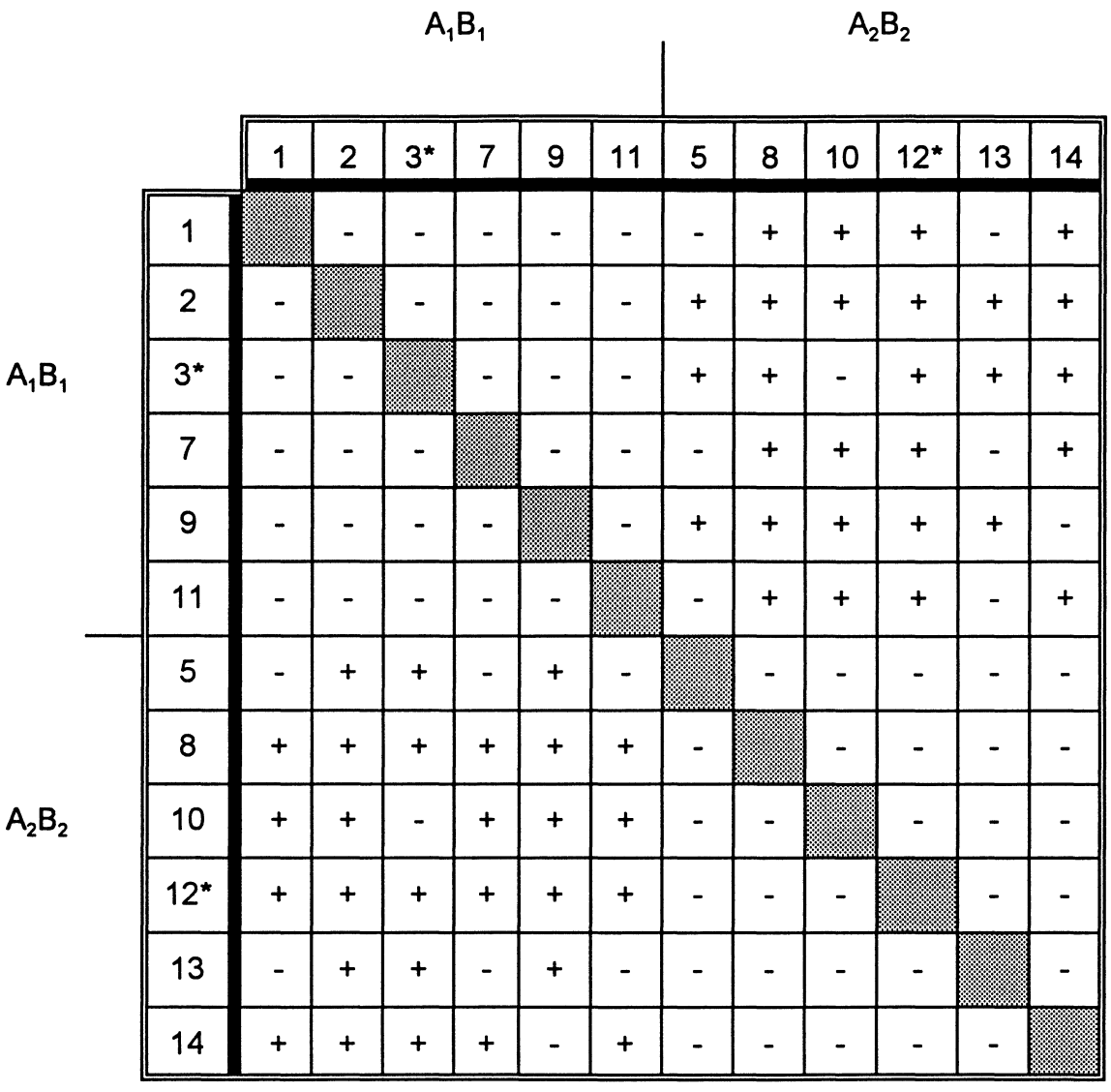


Fig. 10. Self-cross of *Lentinellus angustifolius* ANM 492; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and assigned mating types at top and left.

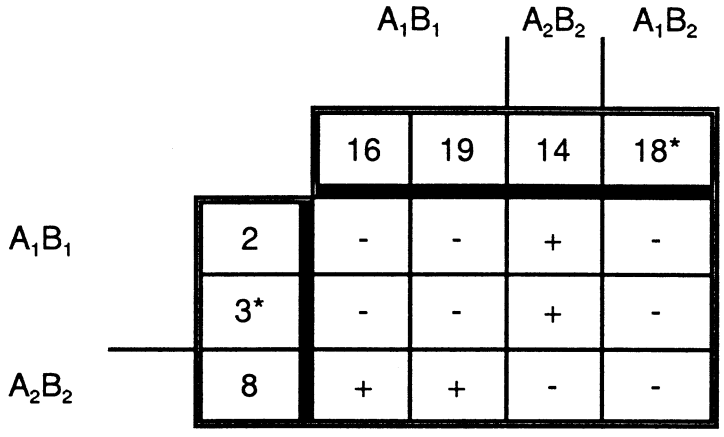


Fig. 11. Additional pairings of *Lentinellus angustifolius* ANM 492 to identify A_1B_2 mating type; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and assigned mating types at top and left.

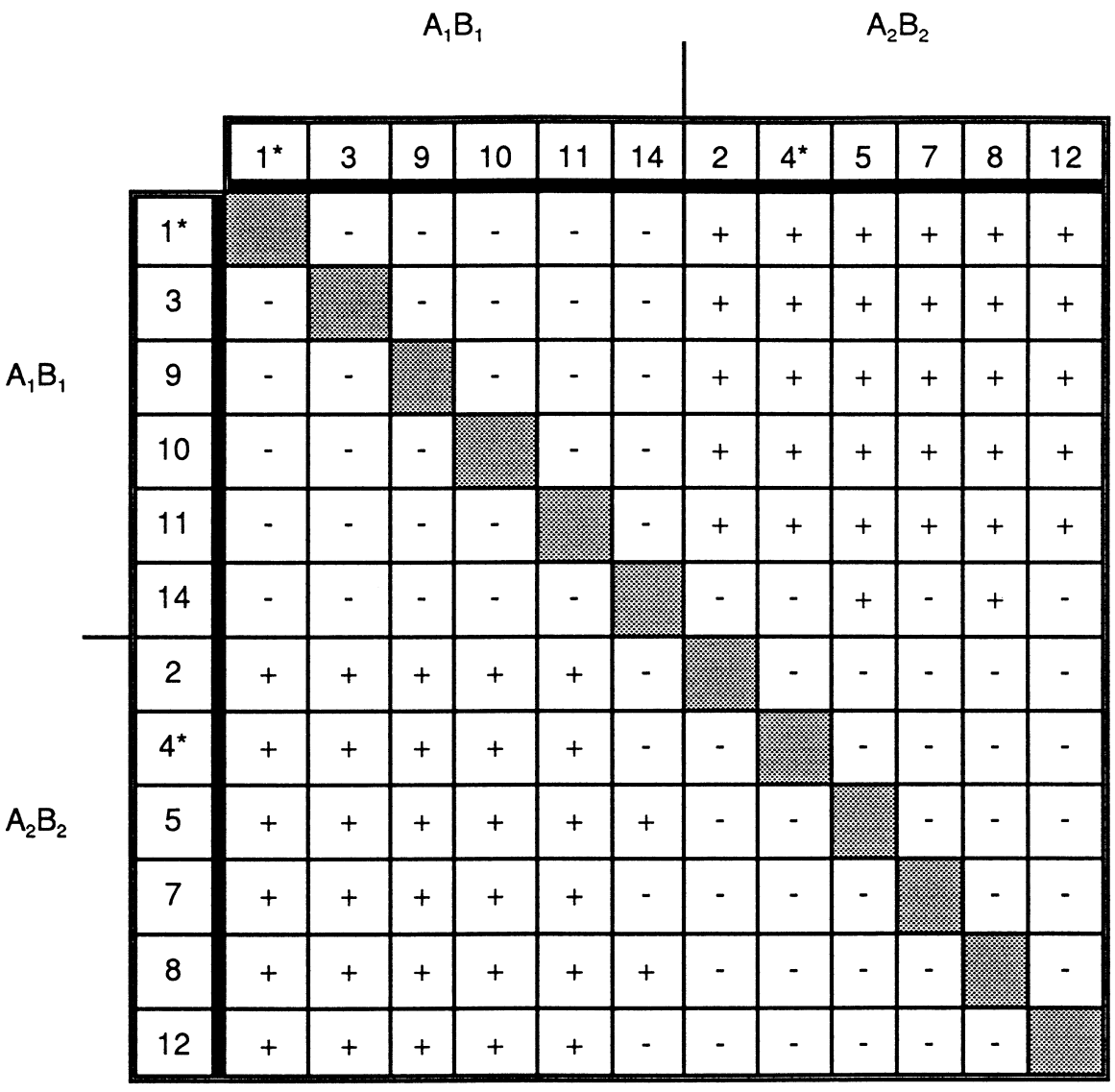


Fig. 12. Self-cross of *Lentinellus angustifolius* ANM 495; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and assigned mating types at top and left.

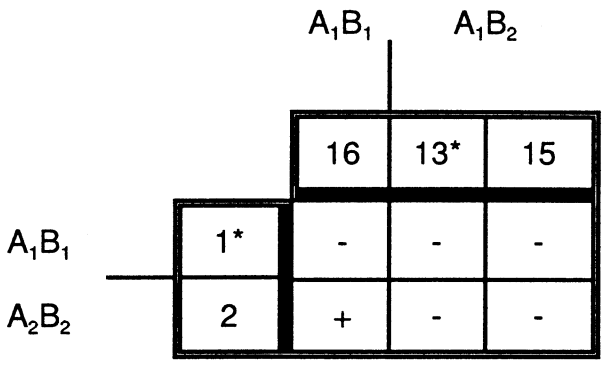


Fig. 13. Additional pairings of *Lentinellus angustifolius* ANM 495 to identify A_1B_2 mating type; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and assigned mating types at top and left.

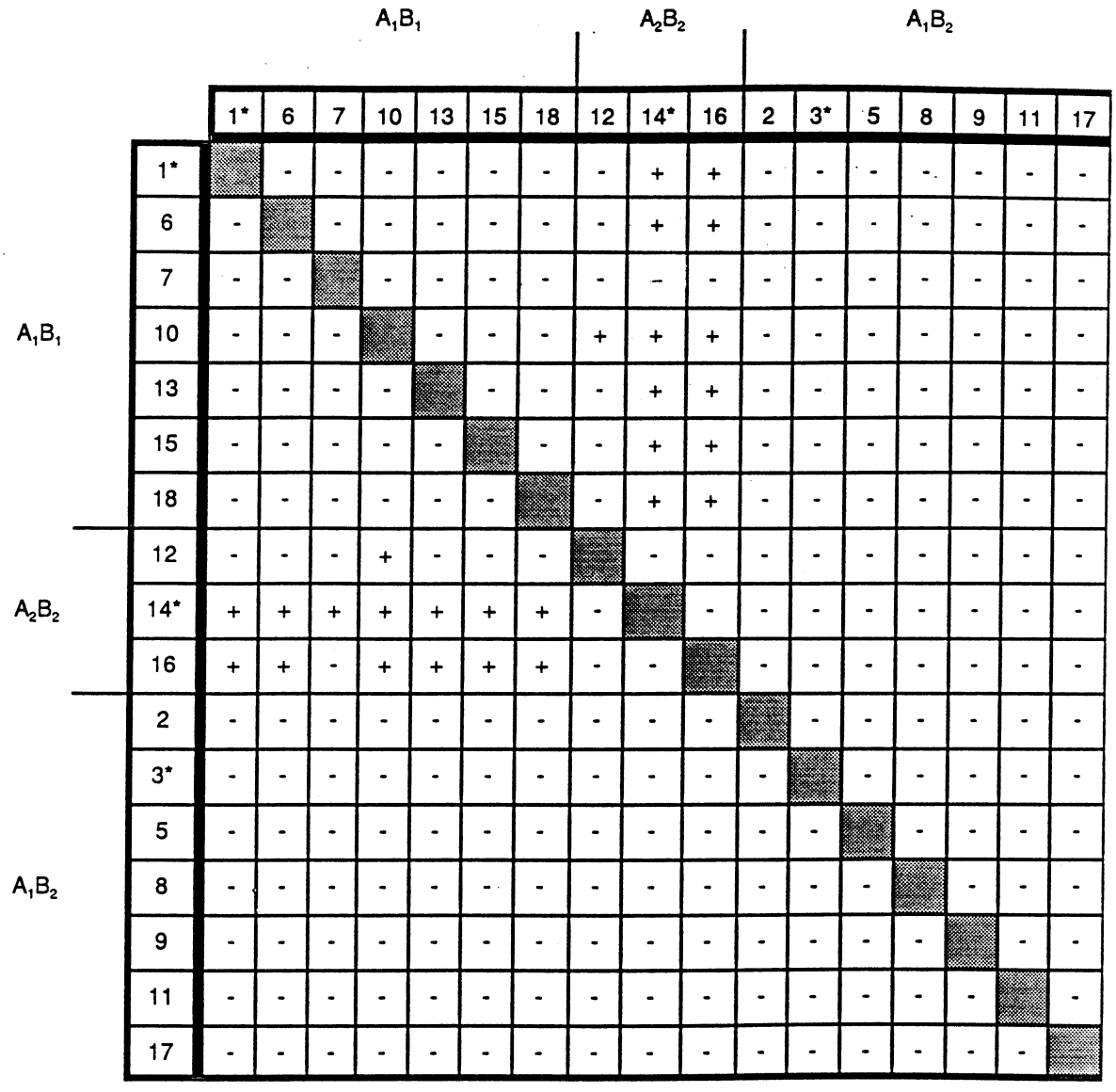


Fig. 14. Self-cross of *Lentinellus angustifolius* RHP 2036; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and assigned mating types at top and left.

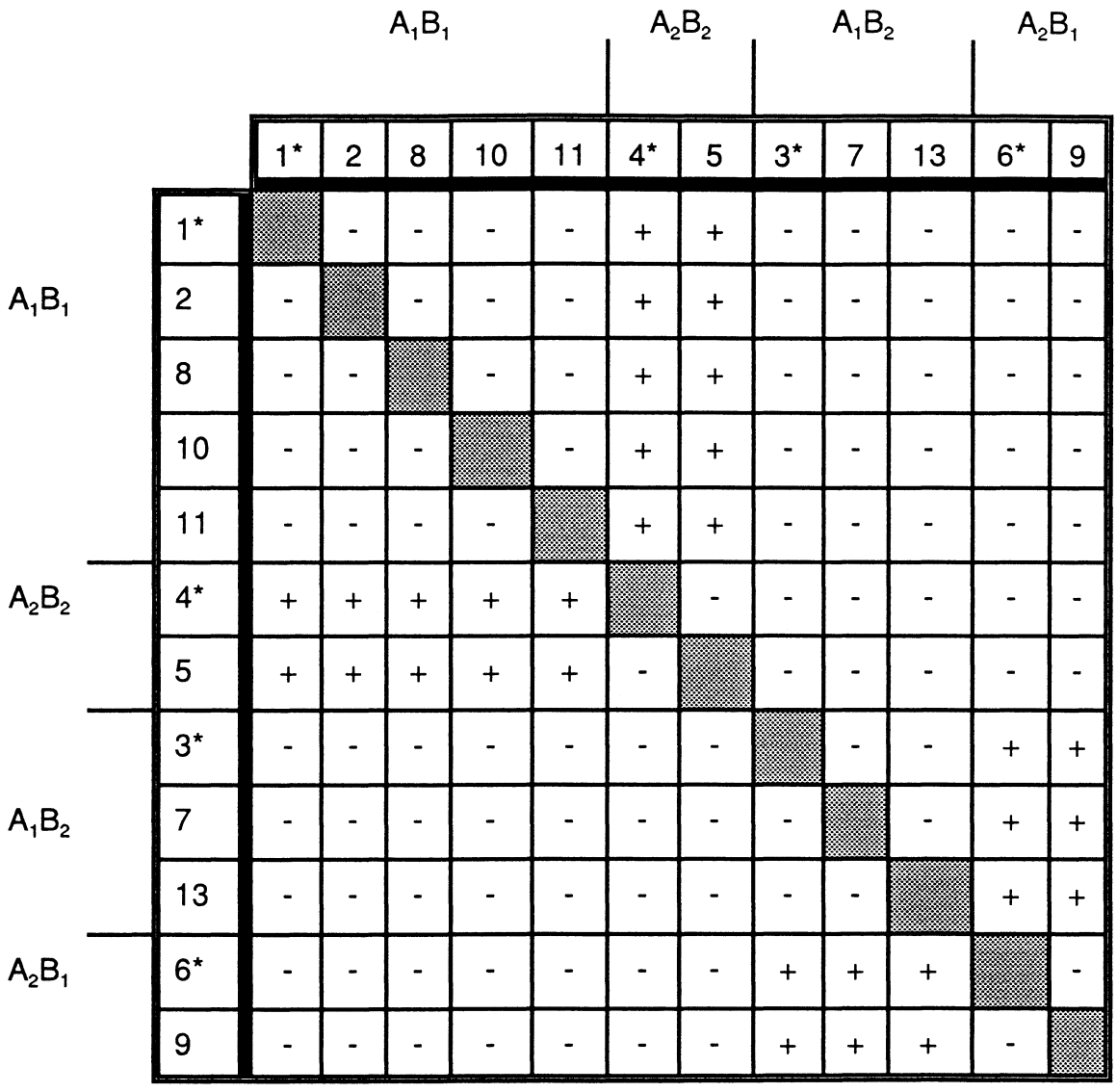


Fig. 15. Self-cross of *Lentinellus angustifolius* RHP 8768; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and assigned mating types at top and left.

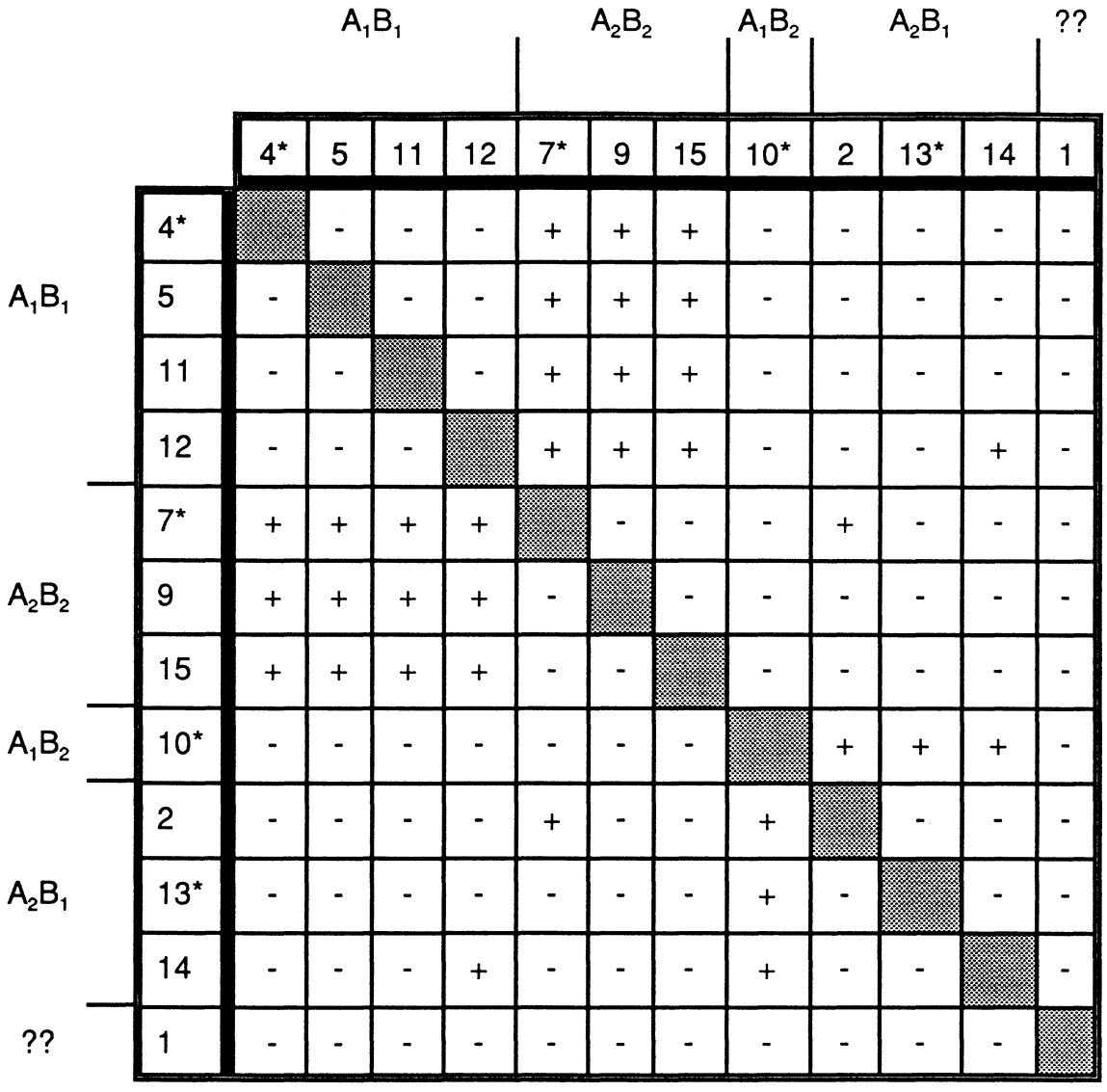


Fig. 16. Self-cross of *Lentinellus vulpinus* RHP 7966; + = compatible mating, - = incompatible mating, * = tester strain. Isolate numbers and assigned mating types at top and left.

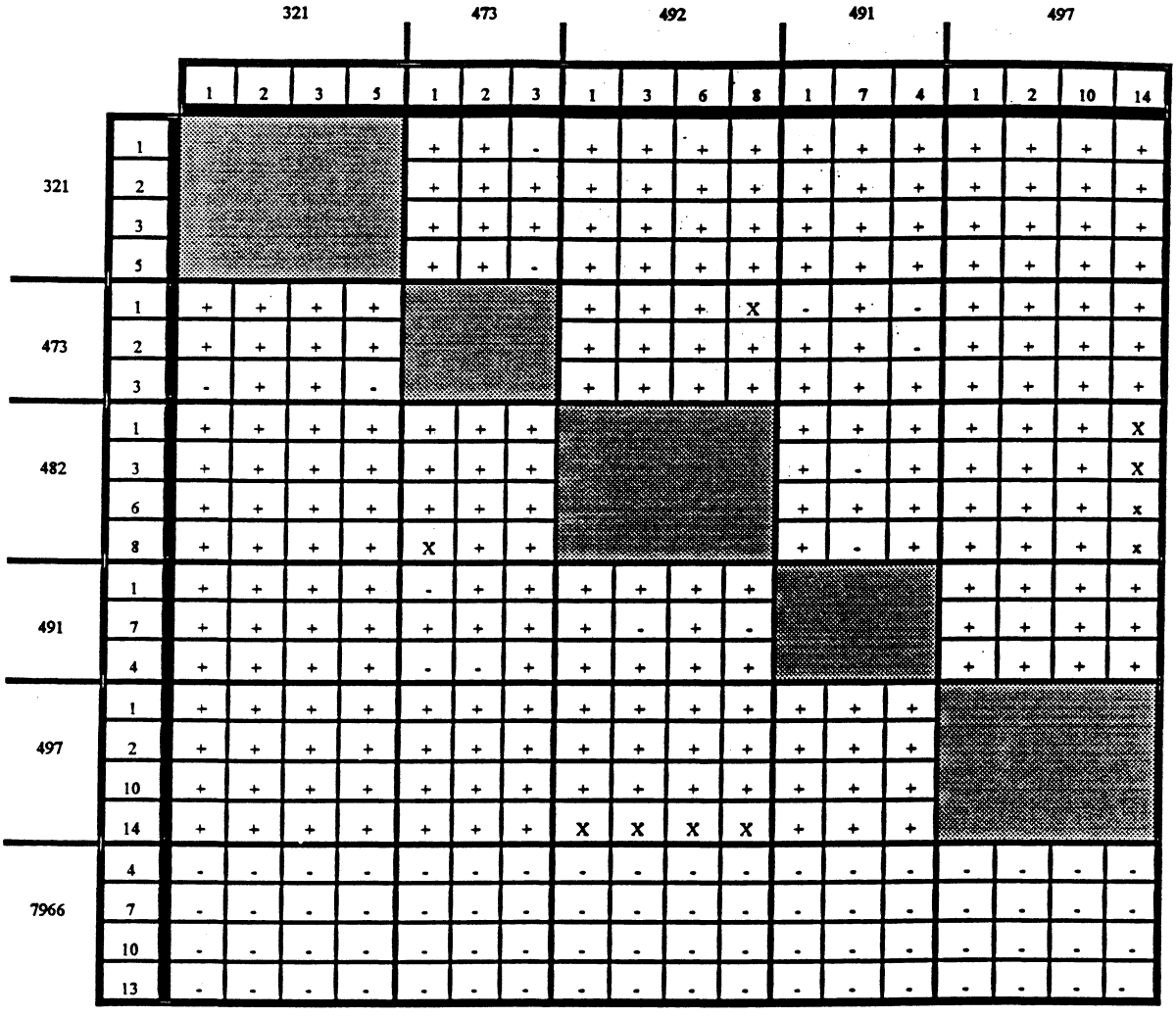


Fig. 17. Mating grid of *L. ursinus* (ANM 321, ANM 473, ANM 482, ANM 491, ANM 497) and *L. vulpinus* (RHP 7966); + = compatible mating; - = incompatible mating; X = contaminated mating. Collection numbers and assigned tester strains are arranged at the top and along the left as A_1B_1, A_2B_2, A_1B_2, A_2B_1, respectively.

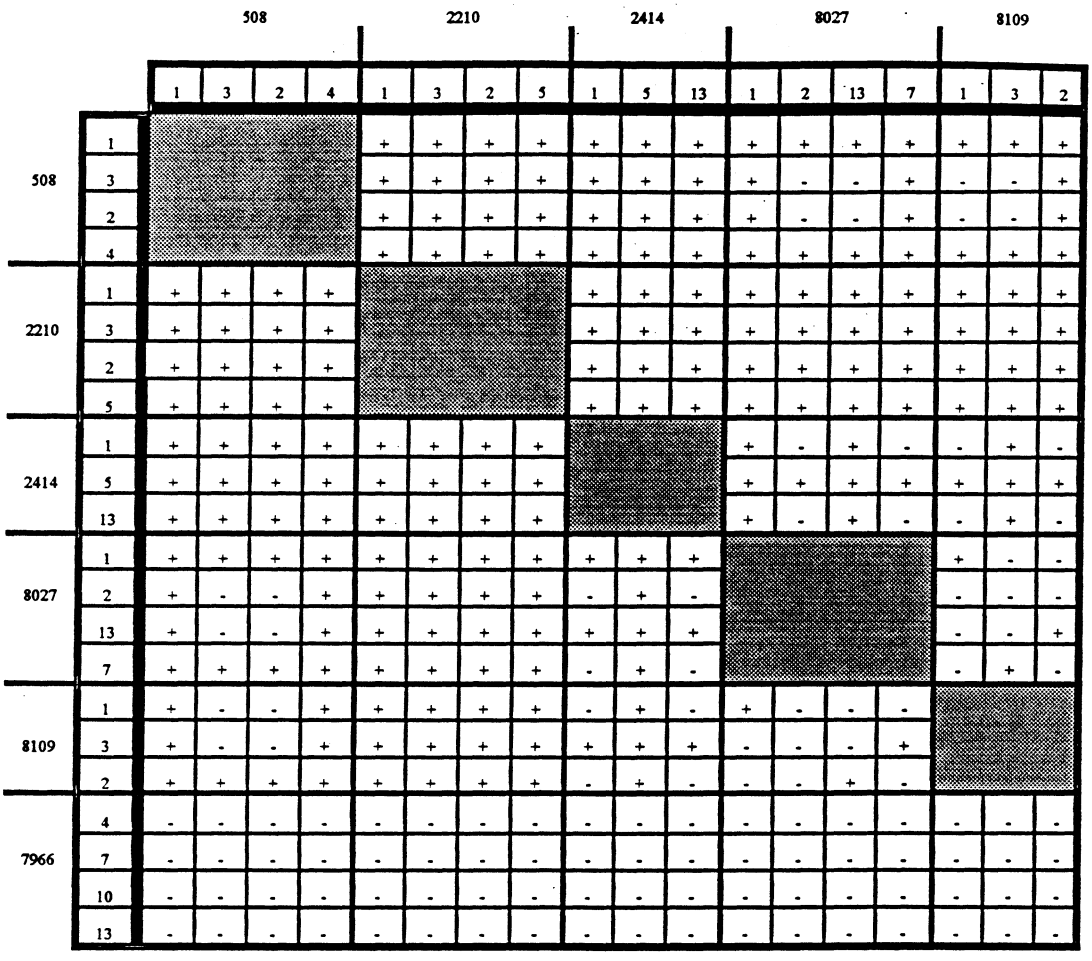
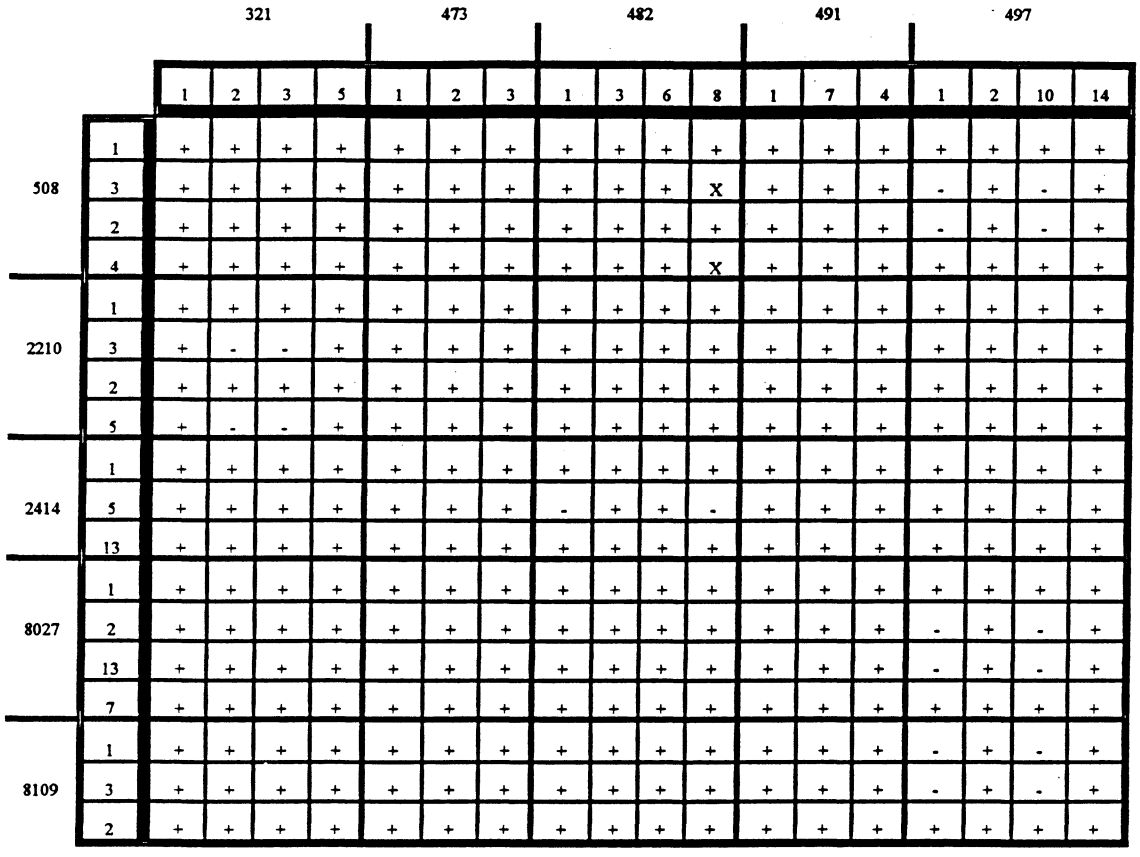


Fig. 18. Mating grid of *L. ursinus* (ANM 508, RHP 2210, RHP 2414, ASM 8027, ASM 8109) and *L. vulpinus* (RHP 7966) collections; + = compatible mating; - = incompatible mating. Collections numbers and assigned tester strains are arranged at the top and along the left as A_1B_1, A_2B_2, A_1B_2, A_2B_1, respectively, except in RHP 2414 which does not contain an A_2B_2 tester strain and in ASM 8109 which does not contain an A_1B_1 tester strain.



		321				473			482				491			497			
		1	2	3	5	1	2	3	1	3	6	8	1	7	4	1	2	10	14
508	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+	+	X	+	+	+	-	+	-	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+
	4	+	+	+	+	+	+	+	+	+	+	X	+	+	+	+	+	+	+
2210	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2414	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+
	13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8027	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+
	13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+
	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8109	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Fig. 19. Mating grid of *L. ursinus* (ANM 321, ANM 473, ANM 482, ANM 491, ANM 497, ANM 508, RHP 2210, RHP 2414, ASM 8027, ASM 8109); + = compatible mating; - = incompatible mating; X = contaminated mating. Collection numbers and assigned tester strains are arranged at the top and along the left as A_1B_1, A_2B_2, A_1B_2, A_2B_1, respectively, except in RHP 2414 which does not contain an A_2B_2 tester strain and in ASM 8109 which does not contain an A_1B_1 tester strain.

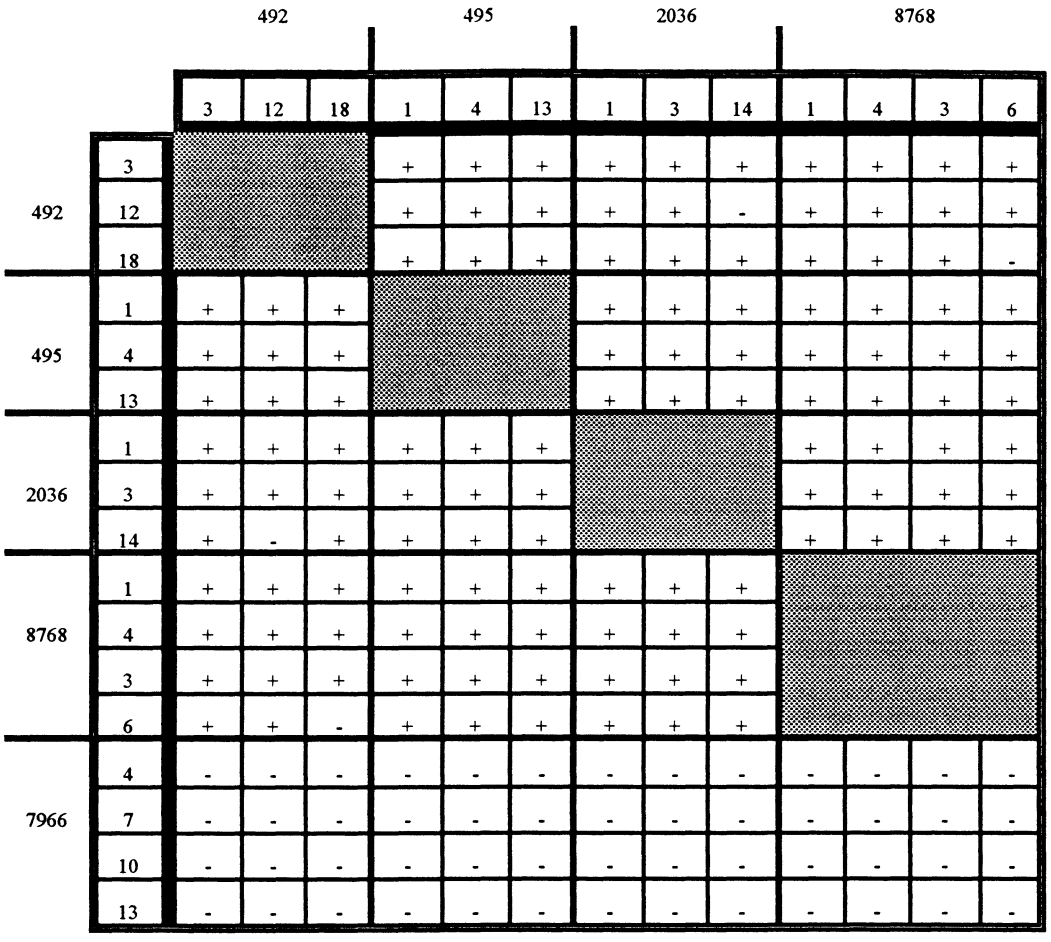


Fig. 20. Mating grid of *L. angustifolius* (ANM 492, ANM 495, RHP 2036, RHP 8768) and *L. vulpinus* (RHP 7966); + = compatible mating; - = incompatible mating. Collection numbers and assigned tester strains are arranged at the top and along the left as A_1B_1, A_2B_2, A_1B_2, A_2B_1, respetively.

		492			495			2036			8768			
		3	12	18	1	4	13	1	3	14	1	4	3	6
321	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-	-
473	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
482	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-	-
491	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-
497	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-	-	-	-	-
	14	-	-	-	-	-	-	-	-	-	-	-	-	-

Fig. 21. Mating grid of *L. angustifolius* (ANM 492, ANM 495, RHP 2036, RHP 8768) and *L. ursinus* (ANM 321, ANM 473, ANM 482, ANM 491, ANM 497); + = compatible mating; - = incompatible mating. Collection numbers and assigned tester strains are arranged at the top and along the left as A_1B_1, A_2B_2, A_1B_2, A_2B_1, respectively.

		492			495			2036			8768			
		3	12	18	1	4	13	1	3	14	1	4	3	6
508	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-
2210	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-	-
2414	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-	-
	13	-	-	-	-	-	-	-	-	-	-	-	-	-
8027	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-
	13	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-	-	-	-
8109	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-

Fig. 22. Mating grid of *L. angustifolius* (ANM 492, ANM 495, RHP 2036, RHP 8768) and *L. ursinus* (ANM 508, RHP 2210, RHP 2414, ASM 8027, ASM 8109); + = compatible mating; - = incompatible mating. Collection numbers and assigned tester strains are arranged at the top and along the left as A_1B_1, A_2B_2, A_1B_2, A_2B_1, respectively, except in RHP 2414 which does not contain an A_2B_2 tester strain and in ASM 8109 which does not contain an A_1B_1 tester strain.

		511				7880			
		1	5	6	7	1	2	3	7
492	3	+	X	X	+	+	+	+	+
	12	+	-	+	+	+	+	-	+
	18	+	+	+	+	+	+	+	+
495	1	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+
	13	+	+	+	+	+	+	+	+
2036	1	+	+	+	+	+	+	+	+
	3	+	+	-	+	+	+	+	+
	14	+	+	+	+	+	+	-	+
8768	1	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
	6	+	X	+	+	+	+	+	+
7880	1	+	+	+	+				
	2	+	+	+	+				
	3	+	-	+	+				
	7	+	-	+	+				

Fig. 23. Mating grid of *L. angustifolius* collections from Austria (ANM 511), Costa Rica (RHP 7880), and North America (ANM 492, ANM 495, RHP 2036, RHP 8768); + = compatible mating; - = incompatible mating; X = contaminated mating. Collections numbers and assigned tester strains are arranged at the top and the left as A_1B_1, A_2B_2, A_1B_2, A_2B_1, respectively.

Vita

Andrew Nicholas Miller was born on December 18, 1972, in Springfield, IL. He was raised in Raymond, a small farming community approximately 40 miles south of Springfield. He attended Lincolnwood High School in Raymond where he was a member of the National Honor Society. He was declared an Illinois State Scholar before graduating in May, 1991.

After receiving an honors scholarship, he enrolled at Lincoln Land Community College in Springfield in August, 1991 and graduated in May, 1993, with an A.S. in Biology. He transferred to Eastern Illinois University in August, 1993, to complete his B.S. in Botany. He received the H.F. Thut Award and the Ernest and Maize Warner Presidential Award in Botany. He graduated magna cum laude with a B.S. in Botany in May, 1995. He continued his education under the direction of Dr. Andrew Methven in the Department of Biological Sciences. He received the Phi Sigma Research Award in Spring 1997 and graduated in Fall 1997.

In January, 1998, Mr. Miller will enroll at the University of Illinois at Chicago, Department of Ecology and Evolution, and continue his study of mycology at the Field Museum of Natural History under the direction of Drs. Gregory Mueller and Sabine Hunhdorf.