

THE GENUS LEUCOSPORIDIUM IN SOUTHERN BRITISH COLUMBIA, AN AREA
OF TEMPERATE CLIMATE

by

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ABSTRACT

A search for members of the genus Leucosporidium (Ustilaginaceae) in and near southern British Columbia has yielded 147 isolates of L. scottii, and a single isolate of an undescribed species with apparent affinities in the genus. L. scottii was primarily found on decaying marine vegetation and driftwood, but isolates were also obtained from stream foam, snow, a decaying turnip root, bark mulch, and rain-derived stem flow over the trunk of a living tree. The species predominated in laboratory incubations of marine algal materials collected in the winter, spring, and late autumn. The majority of isolates obtained directly from natural substrates were also found during periods of cold seasonal conditions. It is suggested that low temperature is characteristic of L. scottii habitats.

Locally-obtained L. scottii strains are all heterothallic, and are completely interfertile with mating test strains originating from the southern hemisphere. Morphological and known physiological and biochemical characters of the local strains are similar to those previously described for the species. However, mating studies of local isolates have revealed that the tetrapolar incompatibility system of L. scottii is distinguished by the possession of multiple alleles at the A locus. Similar modifications of the tetrapolar system are known in Tremella and other heterobasidiomycete genera, but have not previously been reported in species of the Leucosporidium-Rhodosporeidium group.

The strain thought to belong to an undescribed species of Leucosporidium was obtained from a sample of filamentous green algae growing in a vernal pond near a peat bog. The fungus is homothallic, and is morphologically and physiologically distinct from other Leucosporidium and Rhodosporeidium species.

Of the six known species of Leucosporidium, five (L. antarcticum, L. frigidum, L. gelidum, L. nivalis, and L. stokesii) were not found during the course of the present study. All five species are obligately psychrophilic and may not be able to remain established within the study area. However, a survey of local L. scottii isolates showed that 5 out of 147 isolates (3.4%) were obligately psychrophilic.

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I. INTRODUCTION

It has long been known that a diverse group of fungi with basidiomycetous affinities go through a portion of their life histories in a yeast-like budding phase. The first reports of yeast-like budding in a basidiomycete were those of Cornu and Roze (1875) and Cornu (1876). These authors found that the spermatia of certain rusts were capable of "germinating" by budding in the already well-known manner of Saccharomyces cerevisiae. Plowright (1889) confirmed the reports and figured the budding cells. Interestingly, the budding capabilities of rust spermatia are little-known today, despite having received considerable attention in early discussions about the proposed functions of the cells. These discussions were reviewed by Craigie (1931) in his classic paper on rust spermatization.

Soon after the discovery of budding in rusts, Brefeld (1883) discovered the same phenomenon in members of several genera of Ustilaginaceae, including Ustilago. Brefeld carried out thorough and comprehensive studies of the life histories of these fungi, and repeatedly demonstrated the ability of the yeast-like phase to propagate indefinitely on artificial media. He also demonstrated the remainder of the Ustilaginaceous life-history: fusion of yeast-like cells by means of conjugation tubes, formation in host tissues of a penetrating mycelium, production of teliospores by the culminating mycelial phase, germination of the teliospores to produce elongate, septate "hemibasidia", or promycelia, and budding of the sporidia from the promycelia to reproduce the budding phase.

Brefeld thought the above process asexual, and argued that the yeast-like sporidia could not be gametes because gametes were not known to perpetuate themselves asexually. DeBary (1887) argued otherwise, and was eventually vindicated by the cytological studies of Dangeard (1892, 1894). These and related studies showed that budding cells are haploid, and fuse to give a mycelium that is basically dikaryotic, although there may be multinucleate cells. Karyogamy takes place in the teliospores as they form, and meiosis occurs during the process of germination. Products of meiosis are sequestered into each of the four cells of the promycelium. These nuclei then divide in the production of sporidia.

The genus Tremella, primarily known for its gelatinous fruiting body with cruciately septate basidia, was the next fungus shown to produce a yeast phase (Brefeld, 1888). Brefeld showed that the basidiospores of both Tremella and the now-synonymized (Bandoni, 1961) genus Naematelia gave rise to a yeast phase which could be maintained on artificial media. Other members of the Tremellales have since been shown to produce yeast-like phases: Moller (1895) demonstrated this ability for Sirobasidium, while Kobayasi and Tubaki (1965) demonstrated it for Holtermannia and Xenolachne. The recently-described genus Fibulobasidium Bandoni also produces a yeast phase (Bandoni, 1979). Slodki et al. (1966) studied the extracellular polysaccharide production and carbon assimilation patterns of Tremella yeast phases, and concluded that the yeasts were surprisingly similar to the common asexual yeast

species, Cryptococcus laurentii.

Some members of the Auriculariales and the Septobasidiales also produce a budding phase. Mycogloea carnosa Olive produces copious budding cells when germinating basidia are kept under water (Olive, 1950). Donk (1972b) has observed that the figures in which Couch (1938) depicted basidiospore germination in Septobasidium give evidence of the initiation of a budding phase. Kobayasi and Tubaki (1965) have confirmed the existence of a budding phase in Septobasidium as well as in Tjibodasia. Moller's (1895) description of Platygloea blastomyces Moll. also mentions the presence of budding cells.

Kobayasi (1952) and Tubaki and Kobayasi (1971) showed that members of the anomalous family Graphiolaceae, a small group of smut-like fungi, produce an extended budding phase in culture. As with members of the Ustilaginaceae, the budding stage of Graphiola species originates when haploid sporidia are budded from a teliospore which has undergone meiosis (Killian, 1924). The Graphiolaceae have been classed in the order Ustilaginales.

Finally, the Exobasidiales, an order of holobasidiate fungi, contains species which produce an indefinitely-propagating, somewhat yeast-like phase in culture. Branched chains of blastic "conidia" are produced after basidiospores have been germinated in artificial media (Graafland, 1953). These "conidia" continue to reproduce until entire colonies have been formed. Donk (1972a) compared this process to a budding phase.

Nyland (1948, 1949) narrowed the gap between the

basidiomycetes and the conventionally-defined yeast species with his publication of the description of Sporidiobolus johnsonii. The life history of this yeast was described in detail by Laffin and Cutter (1959a, 1959b). S. johnsonii produces a diploid yeast phase which reproduces by budding or ballistospore production. A dikaryotic mycelial phase is subsequently formed. Chlamydospores are formed on the mycelium, and within these structures karyogamy takes place. The diploid phase is regenerated when the chlamydospore germinates. Meiosis in a diploid cell initiates the dikaryophase.

It was not until Banno's (1967) study of Rhodotorula glutinis that a species originally described as an imperfect yeast was shown to have a basidiomycetous phase. Banno demonstrated that the capsulated, carotene-containing haploid yeast cells conjugated to produce a dikaryotic mycelial phase with clamp connections. Terminal and intercalary teliospores formed on the mycelium, and within the teliospores, karyogamy took place. Germination of the teliospores by meiotic division gave rise to a promycelium similar to those of members of the Ustilaginaceae. This promycelium became transversely three-septate, and haploid sporidia budded from each of the four cells. The description of the perfect genus Rhodosporidium was based on these results. Fell et al. (1970) suggested that Rhodosporidium is allied to the Ustilaginaceae.

Fell et al. (1969) found that similar life-cycles occurred in some members of the imperfect yeast genus Candida, the members of which lack Rhodotorula-like carotene. The genus

Leucosporidium was erected to contain the perfect states of six such species. A seventh species, the imperfect state of which had been placed in the Torulopsis grab-bag, was also included in the genus. This species, L. capsuligenum, was recognized as an anomalous element in the genus by Rodrigues de Miranda (1972), and was transferred to the genus Filobasidium (see below).

There are several other published reports which are significant in this area of knowledge. Among these are three studies (Bandoni et al, 1971; Bandoni et al., 1975; Fell and Tallman, 1980) which show the formation of clamped mycelium and teliospore-like structures in matings of Sporobolomyces spp. Recently, two species of Sporobolomyces, S. salmonicolor and S. shibatanus have been shown to have teleomorphs in the genus Sporidiobolus (Fell and Tallman, 1981). Van der Walt (1970a) had earlier published the genus Aessosporon as a putative perfect state of S. salmonicolor, but this genus was based on a misinterpretation (see Fell and Tallman, 1980). Similarly, Syringospora, the proposed basidiomycetous stage of Candida albicans (van der Walt, 1970b) has been disregarded since it was revealed that C. albicans has ascomycetous affinities (see Kreger van-Rij and Veenhuis, 1971; van der Walt and Hopsu-Havu, 1976).

The final major group of dimorphic basidiomycetes is the Filobasidiaceae, a family place by Olive (1968) in the Ustilaginales, and by Cox (1976) in the Aphyllorphorales. Olive (1968) first publicized the existence of the group by

describing the genus Filobasidium, based on the facultative mycoparasite F. floriforme. This species produces a haploid yeast phase, a clamped dikaryotic mycelial phase, and aseptate aerial basidia bearing sessile basidiospores at their apices. Leucosporidium capsuligenum was transferred to Filobasidium after reassessment by Rodrigues de Miranda (1972). The genus Chionosphaera, a fungus similar to F. floriforme but lacking clamps and forming synnematal basidiocarps, was described by Cox (1976). Finally, Kwon-Chung (1978) erected the genus Filobasidiella for the perfect state of the long-notorious pathogenic yeast Cryptococcus neoformans. Filobasidiella has a life-cycle which resembles that of Filobasidium.

The vast majority of the dimorphic basidiomycetes are poorly-known, and the large number of asexual yeasts which have been revealed to have basidiomycetous affinities (Nakase and Komagata, 1971a, 1971b; Bastide et al., 1975; van der Walt and Hopsu-Havu, 1976; von Arx and Weijman, 1978; Hagler and Ahearn, 1981; etc.) suggests that many more exist which are yet unknown. The ecology, genetics, physiology, and biochemistry of a few parasites and pathogens have been extensively studied, but little is known about a diverse collection of saprobic species. In the present study, I selected the genus Leucosporidium for further examination. The genus appears to consist entirely of saprobic forms (Fell et al., 1969).

The six species now recognized in Leucosporidium are: L. antarcticum Fell, Statz., Hunt., & Phaff, L. frigidum (DiMenna) Fell, Statz., Hunt., & Phaff, L. gelidum (DiMenna) Fell,

Statz., Hunt., & Phaff, L. nivalis (DiMenna) Fell, Statz., Hunt., & Phaff, L. stokesii Fell, Statz., Hunt., and Phaff, and L. scottii (Didd. & Lodd.) Fell, Statz., Hunt., & Phaff. These species differ in many ways. Fell et al. (1969) presented a key which distinguished between them on the basis of nutrient assimilation characteristics. However, the species also differ in the morphology of their cells, mycelium, teliospores, and promycelia, in their sexual compatibility systems, and in extracellular starch production and other biochemical characters (Fell et al., 1969; Fell, 1974b); in their physiological ability to cope with environmental heat (Fell et al., 1969); and in their geographic and ecological distribution (most recent review: Fell et al., 1969).

In the present study, I elected to investigate two aspects of the biology of Leucosporidium spp. in greater detail: ecological distribution, and sexual incompatibility system. A summation of published information on these subjects is given below.

The ecological distribution of Leucosporidium spp. must be understood in light of known physiological differences among the species. All the species except L. scottii are obligate psychrophiles; that is, they cannot grow above 19 C. (Fell et al., 1969). L. scottii also contains some obligately psychrophilic strains. Not surprisingly, the five psychrophilic species were all first isolated from polar latitudes.

Three of the five psychrophilic Leucosporidium species had been described as imperfect yeasts before Fell et al. (1969)

set up the genus. L. frigidum, L. gelidum, and L. nivalis were first described as Candida species by DiMenna (1966a). DiMenna (1966b) isolated strains of all three species from Antarctic soil. She also obtained isolates of C. gelida from a soil sample from East Greenland, giving the yeast a known bipolar distribution.

C. frigida has subsequently been isolated only once: Babyeva and Azieva (1980) report obtaining a single strain from tundra soils in West Taimir, U.S.S.R. The isolation of a strain of L. nivalis was reported in the same study. Neither species had previously been known from outside the Antarctic.

The presence of L. gelidum in the northern hemisphere has been confirmed by Babyeva et al. (1976). However, the strains obtained in that study show some dissimilarities with the type strain. Golubev et al. (1977) have reported a single isolate from sap flows of Betula verrucosa (European birch). Finally, Babyeva and Azieva (1980) have isolated yeasts which they believe to be asexual variants of C. gelida from arctic tundra soils.

Little information is available on the distributions of the two Leucosporidium species newly-described in Fell et al.'s (1969) paper. L. antarcticum is primarily known from pack ice regions near the northeastern end of the Antarctic peninsula. (Fell et al., 1969; Fell, 1974a). However, Kockova-Kratochvilova et al. (1972) have obtained an isolate from the fruit of a willow, Salix alba, in East Germany. The second species, L. stokesii, was based on a single strain isolated by

Sinclair and Stokes (1965) from Antarctic snow. No further isolates have been reported.

Compared to the five obligately psychrophilic species, L. scottii appears to be much more widely distributed. Candida scottii, the imperfect state described by Diddens and Lodder (1942), was based on isolates obtained by Scott (1936) in Australia. Scott's isolates were from refrigerated beef and from soil near a meatworks. Clark et al. (1954) isolated a single strain from the surface of a Canadian apple. Numerous isolates were obtained by DiMenna (1955, 1958, 1960a, 1960b, 1966b) from Antarctic, New Zealand, and East Greenland soils, while Kockova-Kratochvilova (1964) and Kockova-Kratochvilova et al. (1965) obtained isolates from plant blossoms and mushroom fruiting bodies, respectively, in Czechoslovakia. Fell et al. (1969) found the species to be abundant in the Antarctic seas (see also Fell, 1974a, 1976). The species was again found in Antarctic soils by Babyeva and Golubev (1969).

After Fell et al. (1969) described the perfect state, L. scottii was found in both temperate and arctic regions. Bruce and Morris (1973) isolated psychrophilic strains of C. scottii from living marine fish in waters near Scotland. Babyeva et al. (1976) and Babyeva and Azieva (1980) reported finding L. scottii in Arctic tundra soils. A study of the yeast mycobiota of the French river Moselle by Hinzelin and Lectard (1978) revealed L. scottii as a minor component. Small numbers of C. scottii isolates have also been found in more-or-less polluted flowing fresh waters by Woollett and Hedrick (1970) and Simard

and Blackwood (1971). The former study was conducted on the Calumet River watershed in Indiana , while the latter involved sampling the yeast mycobiota of the St. Lawrence River in Quebec, Canada. A Leucosporidium isolate obtained from the skin of a hospital patient in by Rose and Kurup (1978) may also be L. scottii.

There is one report of a Leucosporidium strain not belonging to any of the described taxa. The strain was found in tundra soil by Babyeva and Azieva (1980).

It should be noted that numerous reports in the Soviet literature referring to C. scottii as a producer of industrial single-cell protein are based on a misidentification. The strains in question have been identified as strains of Candida mesenterica (Semushina et al., 1974).

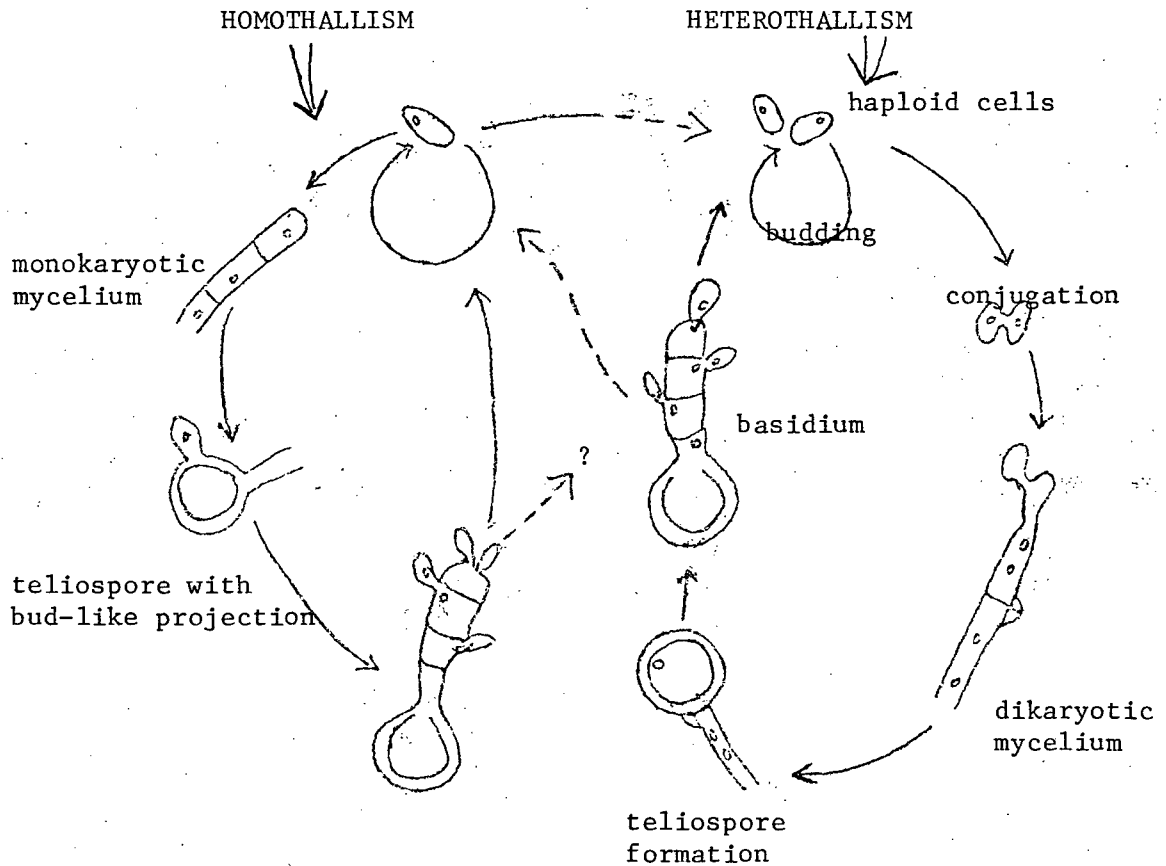
With regard to mating genetics, the genus Leucosporidium is diverse. All species have homothallic (self-fertile) strains, and four species are exclusively homothallic. These species are: L. frigidum, L. gelidum, L. nivalis, and L. stokesii (Fell et al, 1969; Fell 1974b). The observed homothallism is in all cases "primary" (terminology of Raper, 1966); that is, the organism remains monokaryotic throughout its life history. L. antarcticum was believed by Fell et al. (1969) to be exclusively homothallic, but was later shown to have heterothallic strains (Fell, 1974b). These strains possess a biallelic, bipolar mating system (Fell, 1974b) in which the sexual phase is brought about by the conjugation of two compatible mating strains, a and alpha.

Heterothallic strains of L. scottii possess a tetrapolar mating system (Fell, 1974) in which two genes, A and B, each exist in two allelic forms, A_1 and A_2 , and B_1 and B_2 . Strains are compatible when they differ at both loci; that is, an A_1B_1 strain will only mate with an A_2B_2 strain, while an A_1B_2 strain will only mate with an A_2B_1 strain. The life cycle of L. scottii is shown in Figure I.

Published work on mating systems of Leucosporidium species has been the result of study of strains obtained in the southern hemisphere. The incompatibility genetics of northern-hemisphere strains have not been investigated; nor has there been an investigation of the relationship of northern- and southern-hemisphere strains. It has been demonstrated that some fungal species possess distinct populations of strains distinguished only by mutual intersterility (Ullrich, 1973; Anderson and Ullrich, 1979). Whether or not such groups of strains occur in Leucosporidium spp. has not been examined. All that is known is that all southern-hemisphere strains tested have proven to be interfertile within species, and not between species (Fell et al., 1969).

In order to accomplish the aims of the present study, I pursued four lines of investigation. Firstly, I undertook to do a regional monographic study of Leucosporidium spp. in southwestern British Columbia and adjacent Washington. A wide variety of substrates and habitats were sampled in order to permit collection of as diverse a range as possible of Leucosporidium strains. Laboratory culturing techniques were

FIGURE 1: The life cycle of Leucosporidium scottii
(after Fell et al., 1969)



designed to permit detection of new taxa related to Leucosporidium spp. Secondly, in conjunction with the regional monograph, an attempt was made to determine the range of habitats occupied by Leucosporidium spp. in the study area. It was hoped that by means of such a study, insight would be gained into the roles of Leucosporidium spp. in temperate habitats in general. Thirdly, isolates obtained from the study area were screened for psychrophily, in order to determine the ecological distribution of psychrophiles within the area. Finally, the mating systems of locally-isolated Leucosporidium strains were examined, and compared with those of southern-hemisphere strains. A search was made for intersterile groups of strains.

II. MATERIALS AND METHODS

Collection of samples

Samples of various materials were collected and tested for the presence of Leucosporidium spp. All materials, with the exceptions noted below, were collected in sterile plastic bags and returned to the laboratory for further study. Materials tested are listed below; they are grouped according to the habitats from which they originated.

A) Marine environment

1. marine macroalgae

- i) Fucus distichus L.
- ii) Ulva lactuca L.
- iii) Macrocystis integrifolia Bory.
- iv) Desmarestia intermedia Post. & Rupr.
- v) Nereocystis luetkeana Mertens (Post. & Rupr.)

2. seagrass (Zostera marina L.)

3. crab parts

4. driftwood

5. moist beach sand

B) Freshwater environment

1. stream water

2. stream foam

3. submerged lotic mosses (Brachythecium sp.)

4. peat bog water

5. Sphagnum mosses from peat bog

6. decaying peat bog vegetation

7. freshwater green algae from vernal ponds (Ulothrix, Draparnaldia)

C) Terrestrial environment

1. mixed bark mulch and soil
2. bark of standing trees (Populus and Alnus)
3. terrestrial mosses
4. marsh vascular plants (Juncus and Scirpus)
5. fresh mushrooms
 - i) Collybia sp.
 - ii) Melanoleuca melaleuca (Pers. ex Fr.) Murr.
 - iii) Tubaria furfuracea (Pers. ex Fr.) Gillet.
6. decayed mushrooms
7. decayed turnip (Brassica rapa L.)
8. waxberries (Symphoricarpos albus (L.) Blake)
9. spore-drop (Tulasnella sp.)

D) Snow

1. winter snowfall
2. alpine spring and late-summer snowpacks

Precise dates and locations for collections of samples are listed in the 'Results' section of this report.

Most of the marine materials examined were from Georgia Strait, an arm of the north Pacific near Vancouver, B.C. The waters of parts of the strait are brackish in nature, especially during spring runoff. Two of the collecting sites for marine materials, Pt. Grey and Squamish, B.C., were near estuaries. Another site, Orcas Island, Washington, was at a greater distance from sources of fresh water. The final two

marine sites, Pt. McNeill and Long Beach, B.C., were on the open Pacific seacoast.

Marine macroalgae were collected both as attached and as detached specimens, all of which appeared fresh when collected. Detached blades of Zostera were collected from drift, but these blades had also preserved a fresh appearance. Crab parts likewise looked and smelled fresh when collected from moist beach drift. Two driftwood samples were collected, one from an estuarine area and another from the open coast. The wood appeared to have been deposited on the beach immediately before it was collected, and its surface was completely water-soaked. One sample could not be collected in a sterile container, and a non-sterile garbage bag was used. Sub-samples were later taken for further examination from areas which had not come into contact with the bag.

The freshwater materials examined were all from the vicinity of Vancouver, B.C. Foam was collected from streams during periods of rainfall and during dry periods. On one occasion, both dry and moist foam were collected. Otherwise, the foam was moist. Submerged mosses were collected from a stream swollen with rain. It is likely that the area in which the mosses were growing is marshy for most of the year, becoming part of the expanded stream during rains and possibly drying in midsummer. The vernal pond from which the sample of Ulothrix sp. was collected was on the margin of a peat bog.

Samples of terrestrial materials and snow were all collected within B.C. The bark component of the mixed bark

mulch and soil sample was outer bark of Pseudotsuga menziesii (Mirbel) Franco. A water sample was obtained from stem flow over the bark of two standing trees as part of an unrelated study by Mackinnon (unpublished). Rainfall was the source of the water. Terrestrial mosses were sampled after they had been exposed to a prolonged period of daily rainstorms. Dr. R.J. Bandoni supplied a decayed sample of Boletellus zelleri (Murr.) Sing. Snell & Dick apud Snell, Sing. & Dick, and a yeast streak from the surface of a turnip which had been allowed to decay in a field near Vancouver, B.C. in the winter. Waxberries were partly decayed when collected, but were still attached to the shrubs. Yeast colonies which grew among spores shed onto an agar plate by a Tulasnella sp. fruiting body obtained in the wild were incorporated into the study.

A variety of snow samples were collected. Winter snow was from a transient snowfall which had been on the ground approximately ten days and was melting when sampled. The area from which the sample was taken was wooded with deciduous trees. Spring alpine snow was collected from various elevations on Mt. Seymour, near Vancouver. One sample was taken from discoloured snow beneath a Tsuga mertensiana (Bong.) Carr. (mountain hemlock) tree. Several samples of "red snow", snow mixed with red encysted cells of the freshwater Chlorophyte alga Chlamydomonas nivalis (Bauer) Wille., were examined. Late-summer snow was collected from the Wavy Range, Wells-Gray Provincial Park, B.C., approximately 600 km. from Vancouver. Both normal snow and "red snow" were collected.

Winter and spring snow samples were processed in the lab before the snow had melted. The late-summer snow samples were maintained at a temperature below 15 °C. by means of ice packs. Transport and processing of these samples were accomplished within 72 hours.

Enrichment incubations

Bandoni (unpublished) succeeded several years ago in isolating Leucosporidium scottii from a Fucus distichus thallus being incubated in the laboratory in fresh water. In the present study, marine algal samples were subjected to similar enrichment incubations. This technique was carried out in combination with direct sampling of freshly-collected algal materials for yeasts. In the enrichments, algal material was submerged in distilled water and allowed to incubate 3-40 days (usually 10-15) in the lab. In one case, a sample of F. distichus was incubated in filter-sterilized seawater. The results of this incubation were compared with those of fresh-water incubations.

Freshwater algae and bog vegetation were also allowed to incubate in fresh water before sampling for yeasts took place. As with the marine algae, however, some samples were processed directly without incubation.

Incubation temperatures were 5°C. throughout most of the experiment. During the summer months of July and August, however, samples were incubated at 10°C. The light regime consisted of 12 h. light, 12 h. dark.

Streaking of samples

Two kinds of media were used for streaking from sample materials:

- 1) Sorbose-yeast extract medium, containing
 - 4 g/l L-sorbose
 - 0.5 g/l yeast extract
 - 8 g/l ICN agar
 - 0.1 g/l tetracycline
- 2) Cjm (Tremella conjugation medium), containing
 - 2 g/l dextrose
 - 1 g/l Difco soytone
 - 0.5 g/l yeast extract
 - 8 g/l ICN agar
 - 0.05 g/l tetracycline

The former medium is a modification of the sorbose-cellulose medium of Bandoni and Barr (1976). Its sorbose component induces limited, colonial growth in certain Zygomycete and Hyphomycete species which typically have a diffuse, spreading growth form on artificial media (Tatum et al., 1949). The latter medium is a modification of three Cjm media used by Bandoni et al. (1975). Tetracycline was added to both media in order to reduce the growth of bacteria on isolation plates.

Streaks were made directly from sample materials with a wire loop, or by streaking materials themselves on the agar surface. In incubation dishes in which a yeast scum had formed

on the water surface, streaks were made from the scum as well as from the incubated material. Samples containing soil were diluted in sterile water, and streaks were made from the suspensions. In order to increase the diversity of yeast isolates obtained from each sample, the two isolation media were used in combination.

Isolation plates were incubated at 5°C. , except during July and August, when they were kept at 10°C. Light regime was as above.

Selection of yeasts for further testing

When yeasts had formed easily-distinguishable colonies on isolation plates , a sterile loop was used to transfer certain of the strains to new media for further study. Strains were chosen primarily on the basis of appearing to be potential Leucosporidium strains. As the study progressed, I became better able to recognize Leucosporidium strains in culture. However, in order to compensate for previously-unobserved morphological variability and the potential of new taxa, white-coloured yeast strains were widely sampled. Isolation plates from which test strains had been selected were retained for examination a month or more later, when those yeasts remaining in distinct colonies showed full development of colony morphology and pigmentation. Any interesting colonies which had not previously been selected were transferred for further study.

The general-purpose medium used for the transfers was MYPT

medium (Bandoni et al., 1975).

MYPT medium

7 g/l malt extract

1 g/l Difco soytone

0.5 g/l yeast extract

6 g/l ICN agar

0.05 g/l tetracycline

Transfer plates were incubated at 10°C. Yeasts which developed intense carotene pigmentation or dark mycelium were not dealt with further. Colonies visibly made up of more than one yeast type were suspended in sterile distilled water and restreaked for re-isolation.

Storage of yeast stock cultures

Yeasts on transfer plates were subcultured on MYP (tetracycline-free) tubes in order to permit retention over a long period of time. Tubes were stored at 10°C.

Diazonium blue B staining

The diazonium blue B staining technique of Hopsu-Havu et al. (1967) was used to differentiate between ascomycetous and basidiomycetous yeasts. Yeasts with basidiomycetous affinities stain red to purple-red with the reagent, while ascomycetous yeasts do not stain or stain weakly yellow (van der Walt and Hopsu-Havu, 1976). For testing, 1 mg/ml stabilized diazonium

blue B salt was added to a stock solution consisting of 15.8 g/l Trizma HCl buffer (pH 7.0) which had been chilled to 4°C. The staining solution was used immediately, before self-coupling of the diazonium salt could take place. An eyedropper was used to place droplets of solution into yeast colonies on two-week-old transfer plates. Yeasts staining a reddish colour were presumed to be basidiomycetous. Strains showing ascomycetous characteristics were recorded, and these strains were not subjected to further testing in the present study. Yeast colonies in which staining was ambiguous were treated as if they were basidiomycetous.

Basidiomycetous yeast colony morphology

Yeast strains revealed as basidiomycetous by diazonium blue B staining were streaked onto V-8 medium (Wickerham et al., 1946) for development of colony morphology and homothallic sexual stages. The V-8 medium was constituted as follows:

V-8 Medium

200 ml. V-8 vegetable juice (commercial product)
3.0 g. CaCO_3
11 g. ICN agar
800 ml. distilled water

Plates were incubated at 15°C. They were inverted in order to permit detection of ballistospore discharge. After 30 days of incubation, colonies were examined microscopically for the

presence of mycelium, clamp connections, and sexual structures. Asexual strains which had colonial or microscopic characters markedly dissimilar from those of Leucosporidium spp. were not subjected to further study. Those suggestive of Leucosporidium spp. were retained for physiological and genetic testing. Cells from sexually-reproducing colonies were suspended in sterile distilled water and restreaked to separate possible heterothallic mating strains. Single-cell isolates of restreaked strains were transferred to V-8 medium to determine whether or not they would reproduce the sexual cycle.

Inositol assimilation

The ability to assimilate myo-inositol is characteristic of members of the genus Cryptococcus, as well as of some Candida species of basidiomycetous affinities, and of Leucosporidium frigidum, L. gelidum, L. nivalis, and L. stokesii. (see Lodder, 1970). I used this test primarily to quickly sort Cryptococcus spp. strains from L. scottii strains. The medium used for the detection of inositol assimilation was that of Paliwal et al. (1979).

Rapid test for inositol assimilation (pH 7.0)

10 g/l inositol

0.67 g/l Difco yeast nitrogen base

20 mg/l bromocresol purple dye

Fifteen-ml. aliquots of this medium were poured into 125-ml. erlenmeyer flasks. The opening of each flask was sealed with a double-thickness of paper towel; this covering was made secure with an encircling elastic band. Subsequently, the flasks were autoclaved at 15 psi for 15 min.

Sterilized flasks were inoculated with yeast colonies and incubated at 25°C. on a New Brunswick Model G-2 Laboratory Rotator set at 100 rpm. The high incubation temperature was chosen so that strains of the inositol-assimilating Leucosporidium species, all of which are obligately psychrophilic, would not be confused with cryptococci and inositol-assimilating Candida spp. A change in colour of the indicator from purple to yellow was held to indicate inositol assimilation.

In general, inositol-assimilating strains were not subjected to further study. However, those strains whose morphological features were strongly suggestive of Leucosporidium spp. were treated in the manner of other potential Leucosporidium strains. This policy was necessary because of three possibilities: 1) mutant inositol-assimilating strains of L. scottii ; 2) mutant mesophilic strains of L. frigidum, L. gelidum, L. nivalis, and L. stokesii; and 3) previously-undescribed mesophilic inositol-assimilating species

of Leucosporidium.

Crossing

Yeasts revealed by the above techniques as potential Leucosporidium strains were crossed with L. scottii mating types obtained from Dr. J. Fell. Crossing was done on V-8 medium (see above). The potato-dextrose-charcoal medium of Butler and Bolkan (1973) was also tested as a mating medium, but proved to be unsatisfactory. Mating type strains were spotted on plates, and each spot received an admixture of cells of the test strain. An additional spot of the test strain was placed on the same plate to serve as a control. These plates were incubated for up to 5 weeks at 15°C. , and then were examined under the dissecting and compound microscopes for the presence of clamped mycelium and teliospores.

Cultures which failed to mate with the L. scottii mating types were grouped according to their morphological characteristics and crossed amongst themselves. The same media and techniques were used. The purposes of these crosses were: 1) to detect groups of L. scottii strains intersterile with Dr. Fell's strains, which have southern-hemisphere provenances; and 2) to detect other heterothallic species in Leucosporidium and allied genera.

Certain strains which had cultural and morphological characteristics reminiscent of L. antarcticum were crossed with mating types of that species obtained from Dr. J. Fell. Crosses were made on V-8 medium stored at 10°C. Six weeks were allowed

for mating.

Extracellular starch test

Strains of white yeast which produced teliospores spontaneously were tested for extracellular production of starch-like compounds. As Fell (1974b) has pointed out, this character is typical of Cryptococcus species, Filobasidium species and the four inositol-assimilating species (see above) of Leucosporidium. The following medium (Lodder and Kreger-van Rij, 1952) was used to detect starch production:

Medium for detection of extracellular starch production

- 1 g/l ammonium sulfate
- 1 g/l potassium dihydrogen phosphate
- 0.5 g/l magnesium sulfate heptahydrate
- 0.5 g/l yeast extract
- 10 g/l glucose
- 8 g/l ICN agar

Yeasts were inoculated and grown for one week at 15°C. Production of starch-like compounds was then detected with iodine-potassium iodide solution. The technique was tested with a strain of Rhodosporidium ? capitatum in order to ensure that starch-positive species would produce the expected blue-violet colour with IKI.

Fermentation of glucose

Further characterization of teliospore-producing strains was carried out by testing for glucose fermentation. Neither L. scottii nor L. antarcticum can ferment glucose; the remainder of the Leucosporidium species are weak fermenters (Fell et al., 1969). The test was carried out by inoculating test strains into standard Durham tubes containing the following medium:

Glucose medium

18 g/l dextrose

1 g/l Difco soytone

0.5 g/l yeast extract

Tubes were inoculated at 15°C. for 2 days with screw-caps slightly open. The caps were then tightened shut and incubation was continued for a further 15 days. The medium and technique were modifications of those of Santa Maria (1972). Formation of a gas bubble in the insert was held to indicate fermentative activity.

Detection of psychrophilic strains

All confirmed Leucosporidium strains and a number of suspected strains were tested for ability to grow at 25°C. The maximum temperature for growth of obligately psychrophilic Leucosporidium species is generally 17°C. (Fell and Phaff, 1970). Test strains were inoculated onto MYPT medium (see above) and kept at 25°C. for two weeks. Two known psychrophilic

strains, the two mating type strains of L. antarcticum, were tested in the same manner. At the end of the week, colonies showing negligible or weak growth were noted.

III. RESULTS

Description of Leucosporidium strains examined

Of the 629 yeast strains isolated during the course of this study, a total of 147 strains were identified as Leucosporidium scottii. All L. scottii strains were interfertile with the southern-hemisphere mating strains used as testers. In crosses between local isolates and test strains, mating proceeded in the usual manner: clamped mycelium formed around and beneath the colonies, and terminal and intercalary teliospores were produced on that mycelium 3-4 weeks after crossing. The teliospores were hyaline and granular in appearance under the microscope, and were usually 8-10 μ m. in size. On a macroscopic scale, they often formed highly-visible golden-brown masses around colonies where mating had taken place. Occasionally, they were not produced in the area around the mixed yeast colony, but were instead produced on its surface, giving a mottled appearance. Where germination was attempted with teliospores obtained from matings of test strains and local isolates, apparently-normal promycelia were formed.

Locally-isolated L. scottii strains did not assimilate inositol, produce extracellular starch-like material, or ferment glucose. In these respects, the strains were similar to southern-hemisphere strains (see Fell et al., 1969). No comprehensive tests of carbon- or nitrogen-assimilation patterns were done.

Two distinct groups of yeasts with superficial similarity to L. scottii failed to cross with test strains of that species. Strains of each of the two groups were intercrossed, but with negative results. One group was later revealed to contain strains of a Candida species of ascomycetous affinities. This group of strains appeared to stain red in diazonium blue B, but closer examination revealed that the red colour was weak, and was derived from coupling of the dye with components in the medium beneath the colonies. The cells themselves were not stained. One member of this group of strains was tested for fermentative ability at 25°C. and gave a weak positive result. The second group contained inositol-assimilating strains belonging to the genus Cryptococcus.

Also, a total of 44 locally obtained isolates of various descriptions were crossed with mating test strains of L. antarcticum. All results were negative.

Five local isolates produced teliospores spontaneously. Three were later revealed to be mixed cultures containing two compatible L. scottii mating types. A fourth was contaminated with Rhodosporidium ?capitatum. The fifth strain, RCS-Y478, appeared to represent a homothallic species of Leucosporidium, but the strain did not resemble those of any of the previously-described species.

Isolate RCS-Y478 was obtained from a direct streak of a sample of the freshwater green alga, Ulothrix sp. The algal sample had been collected Jan. 8, 1981 in a vernal pond near the margin of Camosun Bog, University of B.C. Endowment Lands,

Vancouver. On V-8 medium, the strain produces off-white, creamy, domed colonies surrounded by a prominent fringe of aerial mycelium, and a band of mycelium developing near the agar surface. The mycelium lacks clamp connections. Yeast cells are budded from the mycelium in whorls around the septa, or in rosettes at the apices of short lateral branches. Teliospores are produced terminally on short side-branches of the mycelium. They are spherical to obpyriform, single or uncommonly double, granular, and hyaline. Their size is unusual: 7-20 X 7-13 $\mu\text{m.}$, as opposed to 7-16 $\mu\text{m.}$ diameter for L. scottii teliospores (commonly 8-10 $\mu\text{m.}$), and 5-7.5 X 5.5-11 $\mu\text{m.}$ for L. frigidum teliospores (Fell et al., 1969). The strain grows at 25°C., ferments glucose, and fails both to assimilate inositol and to produce extracellular starch. This combination of characters is unknown in the genus. Attempts to germinate the teliospores have so far been unsuccessful. Further characterization of this yeast needs to be done before its affinities can be conclusively drawn.

Distribution of L. scottii within study area

Isolates of L. scottii were obtained from a variety of habitats (Tab. I). The largest number of isolates, 123 out of the total 147, were obtained from marine sources (Tab. II). In fact, 52% of all marine white yeast strains examined turned out to be L. scottii strains.

Brown algae (Phaeophyta) were good sources of the yeast. Specimens of Fucus distichus, Desmarestia intermedia, and

Nereocystis luetkeana collected during the fall, winter, and spring months yielded large numbers of L. scottii strains. However, L. scottii could not be isolated from F. distichus and Macrocystis integrifolia samples collected during the summer months. The apparent seasonal occurrence of L. scottii was reflected both in direct streaks from freshly-collected materials, and in selective incubations.

Both freshwater and salt water incubations of F. distichus generated large numbers of L. scottii isolates. Development of L. scottii in such incubations does not appear to be affected by salinity. L. scottii was found in streaks from water-surface scum in incubation dishes as well as in streaks from algal materials.

The green alga, Ulva lactuca, was also a good source of L. scottii isolates. No L. scottii was isolated from U. lactuca thalli collected during the summer months, but a number of isolates were obtained from fall collections.

Streaks from the marine vascular plant Zostera marina produced an abundance of L. scottii isolates. Leaves of this plant were streaked both before and after being incubated in fresh water.

Driftwood from relatively low-salinity areas of the Strait of Georgia, and wood from the high-salinity waters of the open Pacific coast both yielded a diversity of white yeast strains, more than half of which turned out to be L. scottii. The single tested samples of crab fragments and beach sand yielded no yeasts of any kind.

L. scottii was much less frequently found in freshwater habitats than in marine habitats (Tab. III). Ten isolates were obtained in total, all of which came from stream foam. Bog water, freshwater green algae, decaying bog vegetation, and submerged mosses yielded no L. scottii strains. However, note the Leucosporidium sp. strain, RCS-Y478, described above, which was isolated from freshwater green algae in a vernal pond.

L. scottii isolates were obtained in stream foam both in warm and cold seasonal conditions. There was no appreciable increase in numbers of isolates from foam during rainy weather, perhaps indicating that the influx of these yeasts into the stream from terrestrial habitats was insubstantial. A single isolate was obtained from desiccated foam.

Few isolates of L. scottii were obtained from the terrestrial materials sampled (Tab. IV). No isolates were found on fresh and decaying mushrooms, on waxberries, on mosses, or on living and dead rushes and sedges from a marsh. A single isolate was obtained from the trunk of a living deciduous tree. Two good sources of L. scottii isolates were found: a streak made by Dr. R.J. Bandoni from the surface of a rotting turnip yielded five isolates, while five more strains were isolated from mixed soil and Pseudotsuga menziesii bark mulch in a garden. The latter group of isolates was obtained under moist winter conditions.

Of a large number of yeasts isolated from snow (Tab. V), only three were identified as L. scottii. One isolate was obtained from each of: 1) alpine summer snow, 2) subalpine

late-spring snow, and 3) transient winter snow. Neither of the two montane isolates was from "red snow".

TABLE I: Isolation of L. scottii from various environments in
and near southern British Columbia

Provenance of isolates	total # of white yeasts isolated	# <u>L. scottii</u> isolates
marine environment	229	123
freshwater environment	153	10
terrestrial habitats	98	11
snow	149	3
all habitats	629	147

In Table II (opposite) results of selective incubations and of direct streaks are combined. Most samples of marine algae and grasses were incubated in fresh water after direct streaking; however, the yeasts isolated from F. distichus collected 10 Nov. 1980 were obtained from a salt-water incubation.

TABLE II: Isolation of L. scottii from the marine environment
in and near southern British Columbia

Date of collection	location	substrate	total # of white yeasts	# of <u>L. scottii</u> isolates
22 Mar. '80	Squamish R. B.C.	<u>Fucus distichus</u>	7	6
21 Mar. '80	Pt. Grey, near Vancouver	<u>F. distichus</u>	8	6
22 Jul. '80	Squamish R.	<u>F. distichus</u>	28	-
9 Sept. '80	Squamish R.	<u>F. distichus</u>	5	-
3 Oct. '80	Pt. Grey	<u>F. distichus</u>	8	-
2 Nov. '80	Orcas Is., Wash.	<u>F. distichus</u>	8	7
10 Nov. '80	Pt. Grey	<u>F. distichus</u>	14	13
27 Jul. '80	Pt. McNeill, Vancouver Is., B.C.	<u>Macrocystis integrifolia</u>	29	-
2 Nov. '80	Orcas Is., Wash.	<u>Nereocystis luetkeana</u>	26	25
2 Nov. '80	Orcas Is.	<u>Desmarestia intermedia</u>	25	25
8 Aug. '80	Pt. Grey	<u>Ulva lactuca</u>	4	-
3 Oct. '80	Pt. Grey	<u>U. lactuca</u>	9	2
2 Nov. '80	Orcas Is., Wash.	<u>U. lactuca</u>	5	5
2 Nov. '80	Orcas Is.	<u>Zostera marina</u>	10	8
15 Dec. '80	Pt. Grey	driftwood	22	15
22 Feb. '81	Long Beach, Vancouver Is., B.C.	driftwood	21	11
8 Aug. '80	Pt. Grey	crab parts	-	-
3 Oct. '80	Pt. Grey	beach sand	-	-
Totals			229	123

TABLE III: Isolation of L. scottii from freshwater habitats in
southern British Columbia

Date of collection	location	substrate	total # of white yeasts	# of <u>L. scottii</u> isolates
2 Mar. '80	Tincan Creek near Vancouver	stream foam	1	-
20 Jun. '80	Tincan Cr.	stream foam	14	1
26 Jun. '80	Tincan Cr.	stream foam	11	-
4 Jul. '80	Tincan Cr.	stream foam	35	-
5 Sept. '80	Tincan Cr.	stream foam	8	4
5 Sept. '80	Tincan Cr.	dry stream foam	8	1
27 Sept. '80	Tincan Cr.	stream foam	2	-
17 Oct. '80	Tincan Cr.	stream foam	9	-
17 Oct. '80	Tincan Cr.	stream water	1	-
9 Nov. '80	Lynn Cr., near Vancouver	stream foam	19	1
4 Mar. '81	unnamed stream, Vancouver	stream foam	9	3
10 Feb. '80	Burns Bog, Richmond, B.C.	bog water	2	-
8 Jan. '81	Camosun Bog, near Vancouver	bog water	1	-
8 Jan. '81	Camosun Bog	submerged rotting vegetation	15	-
7 Jan. '81	Vancouver	<u>Draparnaldia</u> sp. (Chlorophyta)	6	-
8 Jan. '81	Camosun Bog	<u>Ulothrix</u> sp. (Chlorophyta)	4	-
9 Nov. '80	Lynn Cr., Vancouver	<u>Brachythecium</u> sp. (moss)	7	-
8 Jan. '81	Camosun Bog	<u>Sphagnum</u> spp.	1	-
Totals			153	10

TABLE IV: Isolation of L. scottii from terrestrial habitats in
southern British Columbia

Date of collection	location	substrate	total # of white yeasts	# of <u>L. scottii</u> isolates
12 Oct. '80	Vancouver	poplar trunk	1	1
13 Nov. '80	Vancouver	alder trunk	1	-
7 Jan. '81	Vancouver	<u>Symphoricarpos albus</u> berries	10	-
15 Jan. '81	Vancouver	decaying turnip	19	5
23 Jan. '81	Vancouver	<u>Juncus</u> -- live & dead parts	9	-
23 Jan. '81	Vancouver	<u>Scirpus</u> --live & dead	19	-
26 Jan. '81	Vancouver	bark mulch and soil	13	5
26 Jan. '81	Vancouver	mosses	10	-
1 Oct. '80	Vancouver	decaying <u>Collybia</u> sp.	1	-
14 Dec. '80	Vancouver	decaying <u>Boletellus zelleri</u>	-	-
23 Jan. '81	Vancouver	spore drop <u>Tulasnella</u> sp.	2	-
27 Jan. '81	Vancouver	<u>Melano-leuca melaleuca</u>	3	-
4 Feb. '81	Vancouver	decaying mushrooms	4	-
Totals			98	11

TABLE V: Isolation of L. scottii from samples collected in
southern British Columbia

Date of collection	location	substrate	total # of white yeasts	# of <u>L. scottii</u> isolates
21 June '80	Mt. Seymour near Vancouver	snow	42	1
14 Aug. '80	Wavy Range, Wells-Gray Prov. Park, near Clearwater, B.C.	snow	98	1
16 Dec. '80	Vancouver	snow	9	1
Totals			149	3

Psychrophilic strains

Only five of the L. scottii strains isolated in this experiment proved to be obligately psychrophilic. Almost all isolates grew well at 25°C. Colony appearance at 25°C. was generally flattened, opaque, and semi-glossy. Such colonies were distinctly different from the rounded, creamy, semi-transparent colonies typically seen at 15°C. True mycelium was formed at 25°C. as well as at 15°C.

The five psychrophilic strains all came from marine samples. One was from a sample of Ulva lactuca, two were from Desmarestia intermedia, one was from Zostera marina, and the last was from semi-estuarine driftwood. All were obtained in the fall and winter months. When crossed with testers, psychrophilic strains all mated with strains of mating types A₁B₁ and A₂B₁.

Mating genetics

As mentioned previously, all locally-isolated L. scottii strains were interfertile with mating test strains from the southern hemisphere. In mating new isolates of L. scottii with test strains, it was found that a large number (102 out of a total of 147) of new isolates mated with two of the four known mating types (for an explanation of the species' incompatibility system, see 'Introduction' section of this report). In order to determine whether or not this phenomenon was due to mixed cultures, cells of ten strains were suspended

in sterile water and restreaked. Single-cell isolates were obtained from the streaked plates. When these isolates were crossed with mating test strains, all were again found to be compatible with two of the four testers. In all cases, the test strains which mated with local isolates had differing A alleles, but identical B alleles (see Table VI).

It is apparent that each of the ten local isolates in Table VI contained an A allele which was different from both the A_1 and A_2 alleles. A test was conducted in order to determine whether or not the new A alleles were all identical. The ten local isolates were intermated in all possible combinations; the results of this test are shown in Table VII. Note that if all the new A alleles were identical, no mating of the strains would be expected to occur.

In Table VII, it is clear that there is more than one new A allele. If the A allele possessed by strain 37-3 is arbitrarily designated A_3 , it is apparent that strains 37-3 and 5-1 are A_3B_1 , while 23-4, 24-4, and 59-3 are A_3B_2 . Strains 1-3, 2-3, 3-2, and 30-1 possess A alleles which are neither A_1 , A_2 , nor A_3 . The result for strain 31-3 is anomalous: it mates with only one of the proposed A_3B_1 strains. Since two other $A.B_2$ strains (A. designates an A allele or alleles not assigned a number) mate only weakly with one of the two A_3B_1 strains, it is possible that genetic characteristics other than mating type are affecting the success of mating. Strain 31-3 is morphologically different from the other strains, tending to form low, semi-glossy colonies at 15°C. rather than the more

usual creamy, rounded colonies. I would tentatively suggest that strain 31-3 possessed mating type alleles compatible with those of both A_3B_1 strains, but failed to mate with one strain because of an unrelated genetic incompatibility.

It has not been determined whether or not the A_1 alleles possessed by strains 1-3, 2-3, 3-2, 30-1, and probably 31-3 are identical to each other. However, the A allele of strain 1-3 may be arbitrarily designated A_4 . The number of confirmed alleles at the A locus in L. scottii is thereby increased from two to four.

Two other independent crosses of locally-obtained L. scottii isolates were made, and similar results were obtained on both occasions.

It should be noted that strains mating with both A_1 and A_2 testers were isolated from marine, freshwater, and terrestrial habitats, as well as from snow. There were no strains which mated with both B_1 and B_2 testers.

TABLE VI: Mating of local isolates of L. scottii

strain #	compatible mating test strains	assigned mating type
1-3	A ₁ B ₁ , A ₂ B ₁	A.B ₂
2-3	A ₁ B ₁ , A ₂ B ₁	A.B ₂
3-2	A ₁ B ₁ , A ₂ B ₁	A.B ₂
5-1	A ₁ B ₂ , A ₂ B ₂	A.B ₁
23-4	A ₁ B ₁ , A ₂ B ₁	A.B ₂
24-4	A ₁ B ₁ , A ₂ B ₁	A.B ₂
30-1	A ₁ B ₁ , A ₂ B ₁	A.B ₂
31-3	A ₁ B ₁ , A ₂ B ₁	A.B ₂
37-3	A ₁ B ₂ , A ₂ B ₂	A.B ₁
59-3	A ₁ B ₁ , A ₂ B ₁	A.B ₂

The symbol A. designates an A allele which is neither A₁ nor A₂

TABLE VII: Results of crossing A.B₁ and A.B₂ strains

A.B ₁ strains	A.B ₂ strains							
	1-3	2-3	3-2	23-4	24-4	30-1	31-3	59-3
37-3	+	+	+	-	-	+	+	-
5-1	+	+w	+w	-	-	+	-	-

+ and - symbolize positive and negative mating reactions, respectively. The symbol +w indicates a weak positive mating reaction.

IV. DISCUSSION

Although the genus Leucosporidium is best known from Antarctic (Fell et al., 1969; Fell, 1974a, 1975) and Arctic habitats (DiMenna, 1966b; Babyeva et al., 1976; Babyeva and Azieva, 1980), it is clear that L. scottii, at least, is readily isolated in the northern temperate zone. The proportion of L. scottii isolates obtained in this study (147/629 total white yeast strains examined, or 23.4%) is not representative of the abundance of the species in nature, but the species is evidently common and widely-distributed. Locally-obtained isolates are similar culturally and morphologically to the southern-hemisphere isolates described by Fell et al. (1969). Those biochemical and physiological characters which have been examined -- diazonium blue B staining, inositol assimilation, ability to ferment glucose, production of extracellular starch-like compounds, and, incidentally, elaboration of a urease enzyme as detected by the tests of Seeliger (1956) -- all fit previously-described attributes of L. scottii (see Fell et al., 1969; van der Walt and Hopsu-Havu, 1976). Also, local isolates are completely interfertile with southern-hemisphere strains, which suggests that populations in the two hemispheres are not isolated from one another.

Five of the six described species of Leucosporidium were not isolated during the course of this study. Although four of the species -- L. antarcticum, L. frigidum, L. gelidum and L. nivalis -- have all been isolated in the northern hemisphere (see 'Introduction'), the majority of the reports are from

studies of Arctic tundra soils. Only a single isolate of one of the species, an L. antarcticum isolate reported from the flowers of Salix alba in East Germany (Kockova-Kratochvilova et al., 1972), has been found in latitudes comparable to those of the present study area (i.e., below 55°N. Lat.). The species in question are all obligately psychrophilic and may survive poorly outside of arctic and boreal regions. However, it should be noted that obligately psychrophilic bacteria have been found in marine waters off the coast of Oregon (Morita and Burton, 1970), approximately 500 km. south of the present study area. A small number of obligately-psychrophilic L. scottii strains were isolated from the marine environment in the present study. Moreover, bacteria with growth optima below 20°C. ("psychrophiles" as defined by Inniss, 1975) have been isolated from a wide variety of temperate habitats (Inniss, 1975). It remains possible that some or all of the psychrophilic Leucosporidium species exist within the present study area.

One locally-isolated Leucosporidium strain, RCS-Y478, cannot be placed in any of the described species of the genus. This strain was isolated from a sample of the filamentous green alga Ulothrix sp. collected in a vernal pond near a peat bog. The strain differs from L. scottii and L. antarcticum in the size, shape, and site of formation of its teliospores, as well as in its ability to ferment glucose. From the remainder of the Leucosporidium species, it differs in site of formation and size of teliospores, in its inability to assimilate inositol and to produce extracellular starch, and in its ability to grow

at 25°C..

One previously-described heterobasidiomycetous yeast species bears some similarity to strain RCS-Y478. That species is Rhodosporidium malvinellum, described by Fell (1970) from isolates collected in the southern Pacific and Indian oceans. R. malvinellum produces a creamy-white streak after three days growth on malt agar at 12°C.. This streak, however, acquires a mauve coloration after approximately ten days growth, and "at one month the colour has intensified" (Fell, 1970). Subglobose teliospores are borne terminally on short lateral branches of the mycelium, as in strain RCS-Y478, but they are small: (5.1-10) X 7.1-12) μ m. as opposed to (7-20) X (7-13) μ m. for Leucosporidium sp. Neither species assimilates inositol or produces extracellular starch. Unlike R. malvinellum, strain RCS-Y478 is able to ferment glucose. Finally, R. malvinellum has a biallelic bipolar mating system (Fell, 1974b) and clamp connections (Fell, 1970), while strain RCS-Y478 is homothallic and lacks clamps. The importance of characters related to incompatibility is not stressed, however: L. scottii, for example, has heterothallic strains with clamped hyphae as well as homthallic strains with unclamped hyphae. Some relationship between Leucosporidium strain RCS-Y478 and R. malvinellum is tentatively suggested, but further speculation is idle until the teliospores of RCS-Y478 sp. have been germinated.

The largest number of L. scottii isolates obtained in this study were from the marine environment. Although the most reliable means of isolating large numbers of L. scottii strains

was by carrying out incubations of decaying marine vegetation, numerous strains of the species were also obtained from direct streaks of freshly-collected materials on isolation media.

Incubations of phaeophyte algae often yielded extraordinary numbers of L. scottii isolates. Previous studies on decaying brown algae have not resulted in parallel findings. Capriotti (1962) examined Sargassum thalli from the Miami, Florida area and reported Candida tropicalis, Pichia fermentans, and Trichosporon cutaneum as predominant yeast species. Macrocystis pyrifera (giant kelp) from southern California was found to be a rich source of Metschnikowia zobellii and Candida (Metschnikowia) pulcherrima by van Uden and Castelo-Branco (1963). In the study of Siepmann and Hohnk (1962), Ascophyllum nodosum from the North Atlantic appeared to serve as a habitat for Debaryomyces subglobosus, Trichosporon cutaneum, and Rhodotorula glutinis.

In Japan, Suehiro (1960) isolated yeasts from decaying thalli of a number of algal species. These algae were incubated in fresh seawater in the laboratory at 20°C. before being sampled for yeasts. Sargassum hemiphyllum was the algal species which sustained the largest populations of colonizing yeasts. Predominant species were Torulopsis candida, Candida albicans, C. natalensis (C. sake), Trichosporon cutaneum, and Endomycopsis chodati. Suehiro and Tomiyasu (1962) carried out similar incubations in seawater containing antibacterial agents. The predominant colonizer of phaeophyte species in this experiment was Candida natalensis, although C. tropicalis often

became predominant during the summer months.

Living brown algae, including Sargassum and Padina, were sampled by Roth et al. (1962) in Florida and the Bahamas. The algal thalli were found to be low in numbers of yeasts, although there were large numbers of yeasts in the surrounding waters. The most common species were members of the genus Rhodotorula. Candida parapsilosis and Rhodotorula rubra were isolated from living Fucus vesiculosus thalli by Seshadri and Sieburth (1971). This study was carried out using algae from waters near Rhode Island. The same area was the site of the most comprehensive study to date of yeasts growing on marine algal thalli. In this study, Seshadri and Sieburth (1975) used homogenization and chemical filtering techniques in order to obtain yeast isolates from living thalli of Fucus vesiculosus, Ascophyllum nodosum, Laminaria digitatum, L. longicruris, and several non-phaeophyte species. Large numbers of Candida spp. isolates were obtained, leading the authors to conclude that seaweeds were reservoirs of Candida yeasts. No confirmed isolates of C. scottii were obtained; however, a large number of isolates unidentifiable by conventional means were grouped by a computer-generated cluster analysis. Of these isolates, 14% fell into a group similar to C. scottii, C. capsuligenum, C. melinii, C. mesenterica, and several other Candida species. Unfortunately, some of the species listed, including C. melinii (see Bastide et al., 1975), have ascomycetous affinities. Nevertheless, it is possible that some of these isolates were L. scottii slightly disguised by heterodox assimilative

characteristics. Seshadri and Sieburth did not attempt mating studies.

In the present study, no isolates of L. scottii were obtained from brown algae sampled during the summer months or during the early autumn. The yeast was absent both in direct streaks and in incubations. Since incubation temperature was raised from 5°C. to 10°C. during the summer, it is difficult to compare the results of summer enrichment incubations with those from other seasons. However, the lack of L. scottii in direct streaks made during the summer, as well as in early-autumn incubations, must be regarded as significant.

The green alga Ulva lactuca was a reliable source of L. scottii isolates in the present study, although again the yeast was not found during the summer months. L. scottii has not previously been reported from U. lactuca or related algae. Chlorophytes included in the incubations of Suehiro and Tomiyasu (1962) developed abundant populations of Cryptococcus and Rhodotorula species. Seshadri and Sieburth (1971) isolated Rhodotorula lactosa and Candida parapsilosis from U. lactuca collected near Rhode Island. Further study by Seshadri and Sieburth (1975) revealed that living U. lactuca was colonized by a variety of Candida strains, many unidentifiable, and by several species of Rhodotorula. Finally, Patel (1975) found that U. rigida from the Indian coast was primarily colonized by Candida tropicalis and two species of Saccharomyces, while two species of Enteromorpha were colonized by Torulopsis glabrata and T. candida.

Zostera marina (Zosteraceae), was sampled once during the present study and found to be heavily colonized by L. scottii. Sieburth and Thomas (1973) have shown that Z. marina develops a thick fouling crust whose biomass can rival that of the host. The yeast components of the crust have not been characterized. Other seagrass species undergo similar fouling processes (see Sieburth, 1979, for review). Roth et al. (1962) studied beds of Thalassia testudinum, a subtropical species, and found that waters in the vicinity of these plants were densely populated by Rhodotorula pilimanae and R. rubra. Spartina alterniflora (cordgrass) studied in Louisiana marshlands by Ahearn et al. (1970), was found to be colonized by Pichia spartinae and Kluyveromyces drosophilorum.

It is difficult to formulate any general conclusions about the ecology of L. scottii from the above results. One possible generalization, however, is that temperature is an important factor in the establishment and development of this species on marine vegetation. The known distribution of L. scottii in marine waters can be cited in support of such an assertion. The species is widely known from marine waters in the southern regions of the southern hemisphere (Fell, 1974a), and it has been found in a study of obligately psychrophilic yeasts growing on fish near Scotland (Bruce and Morris, 1973). In the present study, it was isolated from marine vegetation collected during cold seasonal conditions. However, L. scottii has never been found in warm-temperate, subtropical, or tropical marine habitats; also, it has been absent from warm incubations

(specifically, the 20°C. incubations of Suehiro, 1960, and Suehiro and Tomiyasu, 1962). Although many investigators have failed to find L. scottii in northern marine waters (Shinano, 1962; Kriss, 1963; Norkrans, 1966; Meyers et al., 1967; Combs et al., 1971; Yamasoto et al., 1974) and on plant and animal materials from those waters (Siepmann and Hohnk, 1962; Ross and Morris, 1965; Seshadri and Sieburth, 1975), the species may be a cosmopolitan inhabitant of cold marine environments.

The seasonality manifested by local L. scottii populations has previously been observed in populations of marine yeasts. Seshadri and Sieburth (1975) found a complex pattern of seasonal variation in taxonomic composition of yeast floras on marine algae. Interestingly, a group of unidentified yeasts nutritionally similar to L. scottii reached a population peak in midwinter. Suehiro and Tomiyasu (1962) also found seasonal changes in the yeast floras of their aforementioned incubations.

Marine driftwood yielded numerous isolates of L. scottii in the present study. Almost nothing is known about the yeast flora of decaying marine wood (Johnson and Sparrow, 1961, have been the most recent authors to lament this fact.). Unfortunately, the techniques used in the present study do not allow an assessment of whether or not the yeast was participating in the process of wood decay. The density of L. scottii cells in the water to which the wood was exposed is unknown. Nevertheless, it is possible that marine wood serves, directly or indirectly, as a substrate for this yeast. Strains

of an unidentified Leucosporidium species have been shown to assimilate xylose (Biely et al., 1978); thus, L. scottii may be able to profit by association with wood-decay microorganisms. The potential ability of L. scottii to assimilate products of lignin degradation is discussed below.

The ease with which L. scottii was isolated in the marine environment in this study stands in contrast to relatively unrewarding examinations which were conducted with materials from the freshwater environment. Only a small number of strains were obtained from streams. Streams were mainly sampled by gathering foam: the branching structures typical of the pseudomycelium of L. scottii were expected to result in the species being accumulated into foam in the manner of branched aquatic conidia (Iqbal and Webster, 1973). From the results obtained, it would appear that L. scottii is a minor component of the yeast mycobiota of local streams, or enters those streams in small quantities from adjacent terrestrial habitats. Alternatively, the foam-sampling method used may give misleading results about stream populations of yeasts which form branched structures. It should be noted that previous studies in which L. scottii or its anamorph have been found in fresh lotic waters (Woollett and Hedrick, 1970; Simard and Blackwood, 1971; Hinzelin and Lectard, 1978) have given similar results.

The anomalous Leucosporidium sp. strain RCS-Y478 (described above), was isolated from the freshwater green alga Ulothrix sp. in a vernal pond near a peat bog. I have been able

to find only one previous report in the literature about a freshwater green alga having been sampled for yeasts. Cooke and Matsuura (1963), although studying waste stabilization ponds, also isolated yeasts from Hydrodictyon mats in an unpolluted freshwater stream. Unfortunately, the identities of many of the yeast isolates obtained could not be determined. No C. scottii isolates (C. scottii was the only Leucosporidium anamorph then described) or similar yeasts were found. The yeast mycobiota of peat bogs is also poorly known. Lund (1954) examined peat bog soils, and found that the predominant yeasts were Schizoblastosporion starkeyi-henricii and Torulopsis candida.

The distribution of L. scottii in the terrestrial habitats studied is of great interest. Of a variety of habitats examined, only three yielded isolates of the yeast: rotting turnip, bark mulch soil, and rain-derived stem flow over the trunk of a living tree. As can be seen, two of these habitats included the bark of trees. As Leucosporidium spp. and anamorphs are not known from the phylloplane (see, for example, Last and Price, 1969; DiMenna, 1971; Kvasnikov et al., 1974), the presence of L. scottii in stem flow is open to interpretation. L. gelidum has previously been detected in a birch slime flux (Golubev et al., 1977).

The presence of L. scottii in bark mulch should perhaps not have been unexpected. The species has been found in various soils (DiMenna, 1955, 1960a, 1960b, 1966b; Scott, 1936; Babyeva and Golubev, 1969; Babyeva et al., 1976; Babyeva and Azieva, 1980), although only small numbers of isolates have been

obtained outside polar regions. The only association of any of the temperate isolates with plant material is found in the study of DiMenna (1960b). This author found C. scottii in forest litter consisting of leaves intermixed with twigs. Once again, bark was present in the habitat. These results suggest, albeit tenuously, that L. scottii may be associated with decaying bark and woody material.

The mycobiota of bark is very poorly known. Lund (1954) attempted to isolate yeasts from the bark of several tree species in Denmark. He found almost no yeasts present, although Saccharomyces fermentati was discovered on the bark of an elm. Phaff and Knapp (1956) obtained Hansenula minuta from decaying Populus tremuloides bark in California. Decaying wood has also received little attention from yeast ecologists. This situation may change: the recent studies of Blanchette and Shaw (1978) and Blanchette (1979) have revealed that yeasts are an important part of the association of organisms which brings about wood decay. At present, only two studies exist in which yeasts on decaying wood in temperate regions have been identified to species. Shehata et al. (1956) found Candida pulcherrima, C. catenulata, and C. clausenii on decaying wood in California. Also in California, Phaff and Knapp (1956) found Pichia polymorpha on decaying Abies and Pinus logs. Previous to the present study, there had been no examination of the yeasts of bark or wood decaying under cold conditions.

The finding of L. scottii in the decaying surface of a turnip (Brassica rapa) root is unprecedented. There has been

scant work on yeasts in the decay of root crops. The roots of Daucus carota and Beta vulgaris were both sampled for yeasts by Lund (1954). Among the yeasts commonly found were Torulopsis molischiana, T. candida, and Candida parapsilosis. Work on yeasts of the rhizosphere of crop plants has yielded interesting results. Babyeva and Savelyeva (1963) discovered that the number of yeasts around the roots of living cabbage plants vastly exceeded the number associated with other crop plants, including carrots, sugarbeets, and cereals. The cabbage plant, like the turnip, is a member of the genus Brassica. Predominant species in the cabbage rhizosphere were Torulopsis aerea (Cryptococcus albidus var. aerea), and Rhodotorula glutinis. Candida species were conspicuously absent from rhizosphere soils, although they flourished in control soils. A further study of crop rhizospheres by Kvasnikov et al. (1974) revealed that cabbage rhizospheres were mainly inhabited by two Cryptococcus species, Oosporidium margaritifera, Torulopsis candida, and Metschnikowia pulcherrima. These rhizosphere mycobiotas are quite different from the cold-weather decay mycobiota isolated in the present study. The decaying turnips were primarily colonized by L. scottii, Rhodosporidium capitatum, a basidiomycetous Candida species, and a basidiomycetous Trichosporon species.

The final habitat in which L. scottii was found in this study was snow. Only three of 149 isolates from various bodies of snow turned out to be strains of L. scottii. It seems likely that the snow sampled did not support indigenous populations of

this yeast. None of the strains isolated from snow was obligately psychrophilic. DiMenna (1955b) has recorded the isolation of a C. scottii strain from air inside a building; such airborne propagules probably account for the occasional appearance of L. scottii in the snow from which I took samples. The isolation of an L. scottii strain from dried stream foam in this study points to at least one mechanism by which the yeast may become airborne. Breaking waves at sea are also capable of releasing large numbers of yeast propagules into the atmosphere (Schlichting, 1974). L. scottii might therefore be expected to be a common component of the airborne yeast mycobiota in coastal regions of B.C.

It is difficult to suggest what factors, if any, typify an L. scottii habitat. One common feature possessed by most of the habitats in which the species has been found is low temperature. Since yeast ecologists in general, myself regrettably included, have been remiss in measuring the temperature of natural yeast substrates, precise information cannot be given on this point. The seasonality of L. scottii on decaying marine vegetation in this study is highly suggestive of an ecological requirement for low temperature, but further work is needed before a generalization about this species can confidently be made.

The selection of L. scottii in incubations of brown algae offers another potential generalization. It is apparent that fresh water does not provide the selective factor in the incubations, although this is what was supposed when the study

began. L. scottii predominated in a salt water control incubation, and hence may not be greatly affected by salinity under enrichment conditions. Temperature may be the sole selective factor, but a second possibility is that the yeast is selected for by the presence of phenolic compounds. Phaeophytes release large quantities of yellow-coloured materials into surrounding waters (Craigie and McLachlan, 1964); these materials consist of tannin-like polyphenols (Haug and Larsen, 1958). Many yeasts appear to be inhibited by these compounds (Seshadri and Sieburth, 1971). Water in brown algal incubations in the present study rapidly became yellow-brown. The abundance of L. scottii in these incubations suggests that it is either insensitive to the compounds, or is capable of reaping some benefit from their presence.

Numerous studies have shown that yeasts are capable of assimilating phenols, benzoic acid compounds, flavonoids, and lignin breakdown products (Henderson, 1961; Harris and Ricketts, 1962; Westlake and Spencer, 1966; Cain et al., 1968; Spencer et al., 1971; Mills et al., 1971; Neujahr, 1978; Pinto et al., 1979). The abilities of Leucosporidium spp. to assimilate these aromatic compounds have never been tested; however, closely-related Rhodosporidium anamorphs are capable of metabolizing a wide range of the compounds. For example, Rhodotorula glutinis, the anamorph of Rhodosporidium toruloides, uses various flavonoids (Spencer and Gorin, 1971).

In the present study, many of the habitats in which L. scottii isolates were obtained in large numbers were habitats

in which such compounds may have been present in relatively high concentrations. In particular, brown algae, decaying marine wood, and bark mulch can be expected to have a high content of aromatic compounds. Other L. scottii habitats, like decaying turnip and decaying Ulva and Zostera, probably also contain such compounds: tannins, especially, are known to be associated with dying and dead plant cells (Goodwin and Mercer, 1972). Of the low-temperature habitats in which L. scottii was infrequently or never found, there are few in which large concentrations of phenolics would be expected.

It is evident from studies of L. scottii in pelagic marine waters (Fell, 1974a) that high concentrations of aromatic compounds cannot be a prerequisite for the survival of the yeast. These compounds may, however, provide an essential reduction in competition in some habitats. In the artificial, enclosed environment of an incubation vessel, this effect may be witnessed in a particularly dramatic form. Alternatively, the compounds may provide a nutrient source which is unavailable to other species at L. scottii's ecological temperature optimum. Tests to determine whether or not L. scottii can assimilate tannins, phenols, and lignin breakdown products would clearly be of interest.

Despite the apparent association of L. scottii with low temperatures in this study, only a small fraction (5/147) of the total isolates were found to be unable to grow at 25°C. All obligately psychrophilic strains came from decaying marine vegetation and wood. Although 90% of the seas' water volume is

below 5°C. (Morita, 1975), it is likely that this stability does not benefit the yeasts present in the much less stable intertidal zone (Seshadri and Sieburth, 1975). The deep waters of the open sea may serve as a reservoir of obligately psychrophilic L. scottii strains, but most colonizers of decaying intertidal or subtidal materials are probably indigenous facultatively psychrophilic strains. This would seem particularly likely in areas as temperate as the present study area.

Although locally-isolated L. scottii strains were interfertile with southern-hemisphere mating test strains, it would appear that many possessed mating alleles not found in those strains. The designations A₃ and A₄ were assigned to two distinct alleles at the A mating locus which were compatible with both A₁ and A₂ testers in crosses. Other A alleles may also have been present. Although the presence of the new mating alleles was only conclusively demonstrated in ten locally-obtained isolates, many more undoubtedly possessed A alleles which were neither A₁ nor A₂. A total of 102 out of 147 L. scottii strains mated with both A₁ and A₂ testers. The exact proportion of these strains which possessed the new alleles is uncertain: some of the strains may have been mixed cultures, rather than the progeny of single-cell isolates. However, the small number of strains which underwent spontaneous mating (3/147) suggests that the number of mixed cultures was low. Even the observed spontaneous matings may have been the result of the streaking of teliospores onto isolation media. It

appears safe to conclude that L. scottii possesses a feature typical of fungi with tetrapolar mating systems (Whitehouse, 1949) -- that is, multiple mating factors.

The discovery of more than two A alleles in L. scottii renders the incompatibility genetics of this species unique in the Leucosporidium-Rhodosporidium group of species (see Fell, 1974b). The other species in the group possess biallelic or multiallelic bipolar mating systems, or tetrapolar systems in which multiple alleles have not been demonstrated. Multiallelic tetrapolar mating systems are widely known in other fungi: for example, Raper, Krongelb, and Baxter (1958) examined 114 Schizophyllum commune strains, and found 96 distinct A factors and 56 distinct B factors. Whitehouse (1949) has stated that tetrapolar mating systems with the minimum number of alleles would tend to be selected against. It should be expected, therefore, that all tetrapolar mating systems will possess at least one locus with more than two alleles.

The L. scottii incompatibility system is similar to those described for other heterobasidiomycetes. In Tremella mesenterica, for example, conjugation is controlled by a single pair of alleles, A and a, while the formation of a viable dikaryon is controlled by a number of B alleles (Bandoni, 1962). The incompatibility systems of Tremella globospora (Brough, 1974) and Sirobasidium magnum (Flegel, 1976) are similar. Also, Ustilago zeae has a comparable system (Rowell and de Vay, 1954; Rowell, 1955). It appears likely that other heterobasidiomycetous species with tetrapolar incompatibility

systems will also have multiple alleles at at least one locus.

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