Mating systems in the Xerulaceae (Agaricales, Basidiomycotina): *Flammulina*

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Recently published taxonomic circumscriptions for taxa within *Flammulina* encouraged mating studies to confirm or reject these names or taxa. Three categories of pairing experiments were performed: 1) self-crosses of monokaryon isolates of exemplars of various putative taxa; 2) inter-exemplar pairings among exemplar strains; and 3) pairings between exemplars and 87 unidentified strains which were grouped on this basis. Mating experiments could distinguish *F. velutipes* from other taxa, but not among infraspecific taxa of *F. velutipes* (vars. *velutipes*, *lactea*, and *lupinicola*) and monokaryons of all these taxa were partially compatible with those of *F. ononidis*. Likewise, isolates of *F. rossica* and *F. elastica* were partially compatible with one another but incompatible with those of other taxa. All other taxa (*F. mexicana*, *F. stratosa*, *F. populicola*, *F. fennae*) appeared to be genetically isolated. Low levels of interspecific hybridization between *F. velutipes* and *F. populicola*, and between *F. velutipes* and *F. rossica/elastica* were also noted.

Key Words——Basidiomycotina; mating systems; Xerulaceae.

Flammulina has become a popular edible mushroom in Japan and more recently in the United States, under the name *enoki-take*. Because of oxygen deprivation, commercial mushrooms form long stipes and small pilei, and usually present very pale basidiome pigmentation. Whether in commercial production or natural, the name *F. velutipes* has been universally applied, and in most journals exclusively so for all strains in the genus (see Buchanan, 1993).

Recently, attention has been drawn to color forms of *F. "velutipes"* (Kirby and Sleath, 1995) and to the distribution of *F. "velutipes"* in time and geography (Vellinga, 1996). The species has been noted as perhaps involved with a decline in elm in the United Kingdom (Marriott, 1995), as fruiting on agar [see *The Mycologist* 9(4): back cover] and by its relative abundance in nature [see *The Mycologist* 8(3): front cover; *Revista di Micologia* 40(1): back cover].

In addition, many papers have dealt with the physiology of *F. "velutipes,"* from somatic growth (Klán, 1978; Klán and Baudisova, 1990a, b; Klán et al., 1989, 1992; Psurtseva, 1983, 1987; Psurtseva and Denisova, 1982; Psurtseva and Mnoukhina, 1996a, b; Kinugawa, 1993; for an introduction to this literature) to fruiting (Gruen, 1976, 1979, 1982, 1983; Wong and Gruen, 1977; Gruen and Wong, 1982; Haindl and Monzer, 1994; McKnight, 1990, 1992; Monzer et al., 1994; Plunkett,

1956; for an introduction to that literature), to genetics and cytogenetics (Aschan, 1952; Takemaru, 1957a, b, c, d, 1961; Takemaru et al., 1995).

There have been reports, however, which cast doubt on the uniform application of the epithet *velutipes* to all fruitings of the genus. For example, Arnolds (1977) described *F. ononidis* based on German material and Bas (1983) proposed *F. fennae* and later summarized the then-known European taxa of the genus (Bas, 1995). Thus, for some years there have been additional epithets available. Moreover, interesting mating data supplied by Lamoure (1989) and Yokoyama (1991), reported incompatibility or partial compatibility between certain strains.

In a paper submitted separately, a brief outline of morphosystematics in *Flammulina* has been furnished together with correct nomenclature on *F. elastica* (Lasch) Redh. & Pet., proposal of two new species, *F. rossica* Redh. & Pet. and *F. populicola* Redh. & Pet. and a new variety, *F. velutipes* var. *lupinicola* Redh. & Pet. In the same paper, *F. velutipes* has been epitypified to provide a specimen with typical microstructures and mating pattern (Redhead and Petersen, 1999). In two other papers the systematic limits of the genus have been extended to include taxa with gelatinized lamellar trama [i.e., *F. callistosporioides* (Singer) Singer; *F. mexicana* Redh. et al. (Redhead et al., 1999a)] and/or stratified pileus trama [*F. stratosa* Redh. et al. (Redhead, et al., 1999b)]. Through these papers, the number of accepted taxa in the genus has doubled, and it was to test the genetic

efficacy of these morphotaxa that the present study was undertaken.

Using monokaryons then in the University of Tennessee (TENN) culture collection, an early experiment pairing monokaryons of eight collections from scattered locations in the Northern Hemisphere by one of us (RHP) found similar results to those published by Yokoyama (1991). Subsequent conversations between RHP and Scott Redhead (SAR) revealed that SAR had already examined numerous herbarium specimens of *Flammulina*, and had found evidence on which to base additional morphotaxa. A project was conceived in which the genus would be examined from the following directions: morphology (Redhead and Petersen, 1999), molecular biology (Methven et al., 1997, 1999), physiology, culture morphology and enzymology (Psurtseva, unpubl. data), and sexuality/compatibility (this paper).

Previous reports have appeared on mating systems in various genera placed in the Xerulaceae *sensu* Redhead (1987). While tetrapolarity seems universal in the family, various novelties also occur. For example, in *Oudemansiella, O. canarii* (Junghuhn) v. Höhnel appears very strictly amphithallic, with rare production of arthroconidia under stressful circumstances (Petersen and Halling, 1993). In addition, pairing experiments in *O. mucida* (Schrad.: Fr.) v. Höhnel from widely disjunct populations (Russian Far East, Japan, Scotland) showed normal dikaryotization but absence of clamp connections on the dikaryon mycelium (Petersen and Halling, 1993). Likewise, *Xerula radicata* (Relhan) Dörfelt was found to form haploid, monokaryon mushrooms which produce mitotic "basidiospores" all of one mating type (Petersen and Methven, 1994). *Strobilurus* and *Pseudohiatula* may be routinely tetrapolar (Petersen, 1995), as is *Megacollybia platyphylla* (Pers.: Fr.) Kotl. & Pouz. (at least from Europe; Petersen and Gordon, 1994). Twelve species of *Xeromphalina* were found to be tetrapolar (Johnson and Petersen, 1997), but at least two sibling species of *X. campanella* await description (Johnson, 1997). Individual species of *Mycena* have been investigated, but only a few *Mycena* taxa were accepted in the Xerulaceae *sensu* Redhead (1987). Only *Xeromphalina* has been investigated in depth, however, until this report on *Flammulina*. The circumscription of the Xerulaceae is still under consideration.

Materials and Methods

Collections and cultures used: A total of 87 strains were involved in one or more pairing experiments. These strains were obtained from many sources and in several forms (i.e. basidiomata from nature; spore print with no voucher basidiomata; dikaryon culture with or without fresh or dried basidiomata; etc.). An annotated list of collections and cultures used is presented below. Materials have been arranged by country and state (province) of origin, if known. The name under which the material was received almost always was *Flammulina velutipes*. In many instances, that name remained after all analysis, but in some cases that name was changed to

accommodate the results of simultaneous observations of basidiomata (Redhead and Petersen, 1999) and/or mating test results. Correct names (as discriminated by mating experiments) appear in the summary below. In addition to the collections cited below, SAR has examined many more, and these will be reported in a subsequent paper.

The term "exemplar" is used here to denote a collection used as a standard to represent a taxon in "recognition grids." Exemplars in mating studies are not always congruent with nomenclatural type specimens, and, like nomenclatural types may not be a "biological ideal." Cases in which cultural exemplars were congruent with nomenclatural types (including neo- or epitypes; Redhead and Petersen, 1999) are as follows: *F. velutipes* (variety and form *velutipes*) culture exemplar no. 7200 (derived from epitype specimen at K); and *F. velutipes* var. *lupinicola* culture exemplar no. 8078 (derived from holotype at TENN). Cases in which culture exemplars were incongruent with nomenclatural types are: *F. populicola* (culture exemplar no. 7271 from Sweden, nomenclatural type DED 6115 from California at SFSU); *F. ononidis* (culture exemplar from CBS fruited to produce monokaryons; holotype specimen at L); *F. elastica* (culture exemplar 7303, later 9004, monokaryons derived from spore prints; isotype specimen at BPI); *F. rossica* (culture exemplar 8281 from Alaska; holotype specimen from Russia at TENN); and *F. fennae*, culture exemplar 8252 from Switzerland, holotype from The Netherlands at L). In all cases except *F. fennae* (see under Discussion below), mating experiments proved that all culture exemplars represented appropriately named taxa.

Below, the form in which the strain was obtained appears in boldface, with the following abbreviations: **NAT**=basidiomata collected in nature, cultures established following procedures by Gordon and Petersen (1992); **SPR**=only spore print received, single-basidiospore isolates derived by spore dilution methods; **BAS-S**=basidiomata and spore print received; **DIK**=only dikaryon culture received, single-basidiospore isolates derived from basidiomata produced in the laboratory, or single-conidial isolates harvested from dikaryon mycelium (see below under Discussion); **SBI**=single-basidiospore isolates; **SCI**=single-conidial isolates derived from dikaryon culture. In addition, many resulting dikaryon strains were fruited under laboratory conditions using methods modified from those by Stamets (1993). Two workers were involved: **fNP**=fruited by Dr. N. Psurtseva; **fASM**=fruited by Dr. A. S. Methven.

Flammulina fennae: NETHERLANDS, The type specimen is not cited here because it was not used in pairing experiments (see below under Discussion). It will be cited in papers on morphotaxonomy and molecular biology. RUSSIA, St. Petersburg, X.87, on hardwood stump, LE-882 (Komarov Inst.) (**DIK SCI**). SWITZERLAND, Canton Graubünden, Resgia, Ramosch, 16.X.95, coll. & det. E. Horak, on *Alnus incana*, no. 8252 (**NAT-S** "Horak;" TENN 54172; **fNP** TENN 54694; **fASM** TENN 54695; **EXEMPLAR** of *F. fennae*);

Flammulina mexicana: MEXICO, Est. Tlaxcala, slopes

of Vulcan Melintzin, 11.VII.93, coll. RHP, J. Cifuentes, A. Estrada-Torres, on dead *Senecio cineraroides*, no. 6237 (**NAT** holotype TENN 52894).

Flammulina ononidis: GERMANY, misit Lehman, CBS 172.82 (**EXEMPLAR** of *F. ononidis*), (**fASM** TENN 54743; **fNP** 54744, **SBI**).

Flammulina populicola: CANADA, Manitoba, N end of Riding Mt. Nat. Park, 24.VIII.79, coll. & det. S. A. Redhead, Redhead no. 3031, DAOM 180395 (**DIK fNP** TENN 54732); Saskatchewan, location and date unknown, misit G. Michalenki, on *Populus tremuloides*, IFO 30490, Shiga Univ. no. 101 (**DIK SCI fNP** TENN 56012), duplicate of "Gruen;" Saskatchewan, location unknown, donated by Dr. H. Gruen (**DIK fASM SCI**, "Gruen," TENN 56019; **fNP** TENN 54776). SWEDEN, Uppland, vic. Uppsala, Gottsundabergen, 7.IX.94, coll. H. Knudsen, no. 7271 (**NAT** TENN 53636; **fNP** TENN 54774; **EXEMPLAR** of *F. populicola*); location and date unknown, rec'd 1963, misit H. Gruen, IFO 7777 (Gruen 3–6; obtained from K. Aschen-Åberg; see also "Gruen 3–6;" **DIK fNP** TENN 54777); Västergotland, vic Trollhatten, 21.IX.91, coll. L. & A. Stridvall, no. 4244 (**NAT** TENN 50580; **fNP** TENN 54779); Uppland, vic. Uppsala, Gottsunda-bergen, 7.IX.94, coll. unknown, ?on hardwood, no 7278 (**NAT** TENN 53632; **fNP** TENN 54734); Uppland, vic. Uppsala, Fyby Urknog, 8.IX.94, coll. RHP, on leaf mold or buried wood, no 7291 (**NAT** TENN 53592; **fNP** 54730); location unknown, date unknown, misit K. Aschen-Åberg to H. Gruen, Gruen no. 3–6 (**fNP** TENN). UNITED STATES, Alaska, Anchorage, Valley of the Moon Park, 17.IX.95, coll. J.E. Johnson & RHP, on ?*Betula*, no. 8195 (**NAT** TENN 54170); Anchorage, Eid Ski Area, Abbott Rd., N61°08.395′, W149°44.693′, 17.IX.95, coll. RHP, on *Betula* stump, no. 8196 (**NAT** TENN 54168; **fNP** TENN 54703); Anchorage, Eid Ski Area, N61°08.395′, W149°44.693′, 17.IX.95, coll. K.W. Hughes, no. 8203 (**NAT** TENN 54174;. **fNP** TENN 54773); Anchorage, Pop Carr Park, 17.IX.95, coll. P. Kempton, on *Populus*, no. 8200 (**NAT** TENN 54171; **fNP** TENN 54778); Anchorage, Pop Carr Park, 17.IX.95, coll. RHP, on *Betula*, no. 8202 (**NAT** TENN 54173; **fNP** 54775); Arizona, Coronado Nat. For., Santa Catalina Mts., Mt. Lemmon, 25.VII.72, coll. J. P. Lindsey, FPLM 2072 (**DIK SCI**); Coronado Nat. For., Santa Catalina Mts., Mt. Lemmon, 2.IX.75, coll. H. H. Burdsall, HHB-8447-SP, FPLM 2095 (**DIK SCI**); Piñaleno Mts., Mt. Graham, 13.X.80, coll. C. Spearman & R. L. Gilbertson, on *Populus tremuloides*, Spearman-32, CFMR 2073 (**DIK fNP**, TENN 54696); California, Sierra Co., Round Lake Trail to Gold Lake, 3.VI.91, coll. & det. R. E. Halling, on *Alnus* or *Populus*, Halling 6536, Desjardin culture no. DED 6115 (**fNP** TENN); Colorado, San Miguel Co., Telluride, date unknown, coll. & det. P. Stamets, FVC-1 (**DIK fNP** TENN); southwestern Colorado, location unknown, 8.IX.94, coll. T. Stampfer, on soil or ?buried wood, no 6685 (**BAS-S** TENN 54784); "central Colorado," location and date unknown, coll. T. Stampfer, Stampfer no. 2, no 6686 (**BAS-S** TENN 54785; **SPR** TENN 56046); New Mexico, Santa Fe Co., Santa Fe, date unknown, coll. J. F. Stampfer, no. 6687 (**BAS-S** TENN 54784; **SPR** TENN

56047); Taos Co., Red River, 36°43.22′N, 105°26.50′W, 28.VIII.95, coll. R. E. Halling, no REH-7495 (**NAT** TENN 56165; NY; **SPR fNP** TENN 54782).

Flammulina rossica/elastica: AUSTRIA, Vienna, 2nd Dist., Danube riverine forest, 27.XI.94, coll. I. Krisai-Greilhuber, on *Salix alba*, no 7303 (**BAS-S**, **EXEMPLAR** of *F. elastica*; TENN 54741; **SPR** TENN 56057); same location, same tree, 24.XI.95, same collector, no. 9004 (**AUXILIARY EXEMPLAR** of *F. elastica* (TENN). GERMANY, Eberswald, received from Ukraine, Komarov Bot. Institute culture collection, LE-389 (**fNP** TENN 56024). NETHERLANDS, Leiden, 21.I.1997, coll. E Vellinga, no. 9001 (TENN 54689); same date, same location, no. 9002 (TENN 54742). RUSSIA, Terr. Primorsk, Dist. Ternei, vic. Plastun, Sichote Alin Biosphere Reserve, Vasnaya, bog trail on N shore of side stream, 12.IX.90, coll. RHP, on *Populus*, no. 3232 (**NAT** TENN 49489; **fNP**); Terr. Primorsk, Dist. Ternei, vic. Plastun, Sichote Alin Biosphere Reserve, Vasnaya, 15.IX.90, coll. RHP, no. 3294 (**NAT** TENN 49345); vic. Magadan, vic Klyopka, N59°44′, E151°29′, 10.VIII.95, coll. H. Knudsen, on *Chosenia arbutifolia*, no. 8256 ("Magadan;" **DIK fNP**); Dist. St. Petersburg, Nizhne-Svirskij Reservation, IX.94, on hardwood stump, LE-N (**DIK SCI**); Terr. Primorsk, vic. Vladivostok, coll. Dr. E. Bullakh, Microbiology Laboratory, Eastern Branch, Soviet Academy of Sciences, IX.1994, ("Bullakh," **SPR fASM** TENN); St. Petersburg, IX.78, on *Salix*, LE 0535 (**DIK**, **fNP** TENN 54805). UNITED STATES, Alaska, vic. Seward, Exit Glacier Rd., 60°11.037′N, 149°33.580′W, 14.IX.95, coll. K.W. Hughes & RHP, on *Salix*, no. 8171 (**NAT** TENN 54169; **EXEMPLAR** of *F. rossica*).

Flammulina stratosa: NEW ZEALAND, South Island, Nelson Dist., Lake Rotoiti Lodge, St. Arnaud Peninsula Track, 17.V.94, coll. A. S. Methven, no. 7099 (**NAT**, holotype, TENN 56240).

Flammulina velutipes: ARGENTINA, Prov. Chubut, Parque Nac. de los Alerces, E shore of Lago Menendez, 9.V.96, coll. RHP, on *Lometia hirsuta*, no. 8326 (**NAT** TENN 55995; **fNP** TENN 54821); same location, same date, coll. RHP, on broom tree, no. 8316 (**NAT** TENN 56007; **fNP** TENN 54820); Parque Nac. de los Alerces, S shore of Lago Verde, 8.V.96, coll. M. Rajchenberg, no 8317 (**NAT fNP** TENN 54822); Prov. Rio Negro, Llao Llao, trail from municipal park, 13.V.96, coll. D. Sime, on *Nothofagus*, no. 8359 (**NAT fNP** TENN 54823). AUSTRALIA, Tasmania, Lake Pedder Nat. Heritage Area, 26.V.91, coll. RHP & G. Kile, on standing *Nothofagus*, no. 3915 (**NAT** TENN 50120; **fNP** TENN 56014). AUSTRIA, Upper Austria, Schärding Dist., comm. Engelhartszell, 19.XI.94, coll. H. Voglmayr, on dead *Salix caprea*, no. 6698 (**BAS-S** TENN 54827; **SPR** TENN 56053; **fNP** TENN 56023); Vienna, 2nd Dist., Danube riverine forest, 27.XI.94, coll. I. Krisai-Greilhuber, on *Acer platanoides*, no. 7304 (**BAS-S** TENN 54809; **SPR** TENN 56058; **fNP** TENN 54793); Vienna, 2nd Dist., Danube riverine forest, 27.XI.94, coll. I. Krisai-Greilhuber, on *Populus* sp., no. 7305 (**BAS-S** TENN 54810; **SPR** TENN 56059; **fNP** TENN 54812); same location, same date. coll. I. Krisai-Greilhuber, on *Aesculus hippo-*

castanum, no. 7306 (TENN 55997). BELGIUM, Kapellen, date unknown, MUCL 28144 (**DIK fASM** TENN 54795); Louvain-la-Neuve, date unknown, on *Ulmus*, MUCL-28497 (**DIK fNP** TENN 54796). BELORUS, location, substratum and date unknown, LE-817 (misit Research Inst. Forestry, Byelorus to Komarov Inst.) (**DIK SCI fNP**, TENN 54790); HUNGARY, Plovdiv, Komarov Bot. Inst. Culture collection no. LE 388 (**fNP** TENN 55996). CANADA, British Columbia, Victoria For. Path. Lab, date unknown (rec'd 31.XII.87, misit. Dr. D. Chu), VC-66-6, DAOM 197553 (**DIK fNP** TENN 56018; **fASM** TENN 56008); Ontario, Rockland, date unknown (rec'd. 31.XII.82), coll. J. Philpott, TC2, DAOM 188690 (**DIK fNP** TENN 56012); vic. Maple, date unknown (rec'd. 23.X.46), coll. & det. H. S. Jackson, DAOM 17788 (**DIK fNP**, TENN 55999); "arboretum," 13.X.30, coll. L. O. Overholts, no. F-1598, CFMR 2083 (**DIK SCI fASM** TENN 55998); CHILE, Grand Isla de Chiloe', vic. Anguay, road to Chepu, 10.IV.95, coll. M. Rajchenberg, on *Nothofagus* log, no. 7368 (**NAT** TENN 56016; **fNP** TENN 54808). CHINA, location and date unknown, leg. Dr. Quimio, MUCL 38389 (duplicate of MUCL 31612; **DIK fNP** TENN 54747, 54745); Fujian, date unknown, MUCL 31612 (duplicate of MUCL 38389; **DIK fNP** TENN 54746); location and date unknown, MUCL 31627 (duplicate of MUCL 38390, **DIK fNP** TENN 54798); location unknown, 1993, trans. Dr. Quimio, MUCL 38390 (duplicate of MUCL 31627; **DIK fNP** TENN 54794). CZECH REPUBLIC, Bohemia, Trebou, 1961, on *Corylus*, LE-385 (misit Inst. Microbiol., Prague, to Komarov Institute) (**DIK** TENN 54805); Prague, date unknown, on *Salix alba*, LE-386 (misit Institute of Microbiology, Prague, to Komarov Inst.) (**DIK, SCI**); Bohemia Hills, Dist. Louny, date unknown (rec'd. 1984), on *Ononis spinosa*, LE 766 (**DIK SCI**). EUROPE, location unknown, date unknown, coll. Roland Treu (**DIK** no RT 766), Virginia Technological Institute & University, VT 2015 (**DIK fNP** TENN 54826). GERMANY, Eberswalde, date unknown (rec'd 23.IV.30), misit Dr. Liese, DAOM 1294 (**DIK fASM** TENN 56013). JAPAN, Tottori Pref., Tottori, Tottori Mycol. Inst. grounds, shiitake-producing grove, 2.X.89, coll. E. Nagasawa & RHP, on *Castanopsis* roots, no 2359 (**NAT** TENN no. 48435); Chiba, Tateyama, V.86, coll. & det. T. Watanabe, no TW 86-1 (**DIK SCI**); Hokkaido, date unknown, on *Alnus hirsuta*, Shiga Univ. no. 115, IFO 30602 (**DIK SCI**); location unknown, IFO 30601, **fASM** TENN 56015. KOREA, location unknown, 1979, Cha no. 1038, IFO 30875 (**DIK SCI**); Haenam, vic. Temple Tachnung, 16.X.84, coll. & det. O. K. Miller, on hardwood stump, Miller no 1576 (VPI herb. no. 21881) (**DIK fNP** TENN 54824). NETHERLANDS, Prov. Zuid-Holland, Schiedam, Beatrix Park, 13.IX.98, coll. G. Fransen-Batenburg, ident. K. Bas, no. 9979 (**BAS-S** L, TENN 57042; as *F. fennae*); Prov. Zeeland, Zeeuws Vlaaderen, Vogelwaarde, 23.XI.81, coll. A. de Meijer, CBS 771.81 (**DIK, EXEMPLAR** of *F. velutipes* var. *lactea*; **fASM** TENN 54748); Leiden, 25.I.1997, coll. E. Vellinga, no. 9005 (**BAS-S** TENN 56031); Baarn, location unknown, 1947, misit Inst. Microbiol. Czechoslovakia, LE-500 (**DIK** TENN 54729). NEW ZEALAND, Forest Research Institute,

1980, coll. Myra Chu-Chou, on *Pseudotsuga menziesii*, Shiga Univ. no. 121, IFO 30905 (duplicate of NZFR-243D; **DIK fASM** TENN 56011; **SCI**); location and date unknown, coll. Myra Chou Chu, NZFR 243D (**DIK SCI fASM** TENN 56001); location, date and substratum unknown, NZFRI-243E (**DIK SCI**). ?PHILIPPINE ISLANDS, "1993," transm. Dr. Quimio, MUCL 38074 (**DIK fNP** TENN 54797). RUSSIA, Terr. Caucasia, slopes of Mt. Aichcha, N43°38.667', E40°26.668', 20.IX.96, coll. RHP, on *Alnus*, no. 8952 (**NAT** TENN 55612); St. Petersburg, 1983, on *Acer*, Komarov Inst. LE-671 (**DIK SCI**); St. Petersburg, X.82, on *Populus*, LE-T (Komarov Inst.) (**DIK fNP** TENN 54789); Pyatigorsk, X.91, on roots of *Carpinus*, LE-P (Komarov Inst.) (**DIK fNP** TENN 54787). SPAIN, Andalucia, Prov. Cadiz, Sierra de Grazalema, Parque Nac. "El Piñapar," 2.IV.96, coll. H. Voglmayr, no. 8391 (**BAS-S** TENN 54728). SWEDEN, location and date unknown, CBS 137.44, G. Lindeberg (**DIK SCI**); Uppland, vic. Uppsala, date unknown, spore print of N. Fries no. 2383, misit Dr. E. Dannell, no. 6682 (**SPR**); Västergotland, Gothenburg, Botanical Institute, 17.IX.91, coll. RHP, on hardwood stump, no 4196 (**NAT** TENN 50673; **fNP**). SWITZERLAND, Zürich, 13.X.94, coll. Dr. O. Holdenriede no. 941002.2, on *Fagus sylvaticus*, no. 6683 (**NAT** TENN 56169; **SPR** TENN 56048). UKRAINE, location and date unknown (rec'd. 1971), LE 0387 (**DIK SCI**). UNITED KINGDOM, Surrey, Ham, Towpath, 3.I.95, coll. P. O. Thomas, on tree by River Thames, det. G. B. Butterfill, no 7200 (basidiomata=K 28262!) (**SPR** TENN 56073; **EXEMPLAR** of *F. velutipes* var. *velutipes*); Surrey, Fetcham Down, The Scrubs, 8.I.95, coll. & det. E. W. Brown, on buried burnt wood among ashes, no. 7318 (basidiomata=K 28264!; culture=KC 592; **SPR** TENN 56070; **fNP** TENN 54816); Surrey, Fetcham Down, 29.I.95, coll. E. W. Brown, no. 7323 (basidiomata=K 28396!; culture=KC 604; **SPR** TENN 56066); Surrey, Chobham Common, 15.I.95, coll. & det. E. W. Brown,, on burnt *Ulex* sp., on blasted heath, no. 7321 (basidiomata=K 28311!; culture=KC 600; **SPR** TENN 56067); Surrey, New Haw, near Weybridge, 28.I.95, coll. & det. G. B. Butterfill, on standing ?*Ulmus*, no 7322 (basidiomata=K 28397!; culture= KC 603; **SPR** TENN 56066; **fNP** TENN 54811); Surrey, date and collector unknown, no. 7323 (basidiomata=K no. 28396!; culture=K 604; **SPR**). UNITED STATES, California, Alameda Co., Hayward, Dry Creek Rd., 31.I.95, coll. Mark Norton, on *Populus*, no 7802 (**SPR** TENN 56074, TENN 54733, TENN 56009); Humboldt Co., McKinleyville, Clam Beach County Park, 24.XI.96, coll. RHP & K. W. Hughes, on *Lupinus arboreus*, no 8078 (**NAT** TENN 55402; **EXEMPLAR** of *F. velutipes* var. *lupinciola*); Humboldt Co., North Jetty, 8.II.95, coll. D. Sime, on *Lupinus arboreus*, no. 7324 (**BAS-S** TENN 54733; **SPR** TENN 56071); North Jetty, 30.XII.94, coll. D. Sime, on dead *Lupinus arboreus*, no. DS 989, no. 7198 (**SPR** TENN 56072; **fNP** TENN no. 56166); Kern Co., Bakersfield, ?10.XII.94, coll. unknown, comm. N. Wilson, no. 7310 (**NAT** TENN 56030; **fNP** TENN 54814); Mendocino Co., Mendocino, ?10.XII.94, coll. N. Wilson, no 7309 (**NAT** TENN 56021; **SPR**); Colorado, vic. Rain-

bow Lake, Mountain Research Station, 1.VII.74, coll. G. Laursen, OKM/GAL no. 11715, VT 0058 (**DIK fNP** TENN 54699); Idaho, Valley Co., Payette Nat. For., 29.VI.82, coll. O. K. Miller, on *Alnus*, VT-1192 (basidiomata: OKM 19865) (**DIK SCI**); Illinois, Clark Co., Rocky Branch, 30.X.96, coll. A. S. Methven, ASM 7647 (**NAT SBIs** by ASM, **fASM** TENN 56026); Du Page Co., Lisle, 18.XI.94, coll. A. Lawrence, no. 6694 (**SPR** TENN 56049; **fNP** TENN 54829); Edgar Co., Baber's Woods, 9.XI.92, coll. A. S. Methven & M. Thon, Thon 293 (**NAT fASM** TENN 56022); Jackson Co., Touch of Nature, 20.X.89, no. 2413 (**NAT** TENN 48545; **fNP** TENN 54799); Macoupin Co., Gillespie, XI.94, coll. A. Lawrence, no 6693 (**SPR** TENN 56050; **fNP** TENN 54831); Moultrie Co., Eagle Creek, 25.XI.94, coll. A. S. Methven, ASM 8022 (**NAT SBIs** by ASM, **fASM** TENN 56025); Iowa, Ledges State Park, 1954, coll. W. E. Eslyn & M. K. Nobles, on *Acer saccherinum*, WEE-SM228-R, FPLM 2078 (**DIK fASM**); location unknown, 1955, on *Acer saccherinum*, WEE-SM816 FPLM 2080 (**DIK SCI**); Michigan, Freesoil, date unknown, coll. P. Christensen, host unknown, no. 6699 (**SPR** TENN 56052); Kalamazoo Co., 19.IX.95, coll. P. D. Olexia ("Olexia-2," **BAS-S** TENN 56028), Mason Co., Ludington, 1994, coll. B. J. Polverento, no. 7301 (**SPR fNP** TENN 54791); Mississippi, location unknown, 15.I.53, on *Acer negundo*, ERT-108-R, FPLM 2076 (**DIK**); New Jersey, Hunterdon Co., 15.XI.94, coll. L. Kudzma no 7316 (**SPR** TENN 56064; **fNP** TENN 54815); Union Co., 15.XI.94, coll. L. Kudzma, no. 7315 (**SPR** TENN 56064; **fNP** TENN 54819); New York, location unknown, coll. M. Larsen & O. K. Miller, MJL-1544-SP, FPLM 2082 (**DIK**); Westchester Co., Pound Ridge, 5.XI.94, coll. S. Scheine, no. 6692 (**SPR** TENN 56051; **fNP** TENN no. 54830); North Carolina, Buncombe Co., Asheville, 21.XI.94, coll. P. Whelihan, on *Fraxinus americanus*, no. 6700 (**SPR** TENN 56055); Macon Co., Highlands, 3.IV.94, coll. RHP, hardwood snag, no. 7020 (**NAT** TENN 56020); Highlands, Chalet Blue, 9.I.93, coll. RHP & K. W. Hughes, no. 6201 (**NAT fNP** TENN 56167); Ohio, between Akron and Cleveland, XI.94, coll. G. Parro, no. 7313, 7314 (**SPR** TENN 56065; **fNP** TENN 54818); Oregon, Multnomah Co., Portland, Forest Park, 2.I.96, coll. S. Redhead & L. L. Norvell, on ?red alder, no. 8278 (Norvell no. 96.01.02; basidiomata=DAOM; **SPR** TENN 56079); Pennsylvania, Carbon Co., Jim Thorpe, 8.XII.94, coll. J. T. Mesaros, on dead *Acer*, no. 7311 (**SPR** TENN 56063; **fNP**); South Carolina, Oconee Co., coll. C. McCleneghan, JC415 (**DIK fASM fNP** TENN 54806); Sumter Nat. Forest, vic. Walhalla, 22.XI.92, coll. S. C. McCleneghan, no. 6171 (**NAT** TENN 52303; **fNP** TENN 56006); Tennessee, Anderson Co., Norris, 25.XII.91, coll. RHP & K. W. Hughes, no 4400 (**NAT** TENN 53165; **fNP fASM** TENN 54800); Knox Co., Knoxville, 14.XI.91, coll. Alex Sloan, no 4631 (**NAT**, TENN 52728; **fNP** TENN 56010; **fASM**); Knox Co., Fort Sanders neighborhood, 11.I.96, coll. RHP, on *Celtis* root, (**NAT** TENN 56004); Knox Co., Knoxville, Martha Washington Dr., 6.XI.86, coll. A. S. Methven, ASM 5026 (**NAT SBIs** by ASM); Sevier Co., GSMNP, grounds of Visitors' Center, 8.I.95, coll. RHP & K. W. Hughes,

no. 7199 (**NAT fNP** TENN 56017); Utah, Salt Lake Co., Salt Lake City, ?.XI. 94, coll. Mark Cannon, on chokecherry, no. 7308 (**SPR** TENN 56062,: **fNP** TENN 54813); Virginia, Montgomery Co., vic. Blacksburg, X.81, coll. G. Reddick, VT 1127 (**DIK fNP** TENN 54825); Virginia, Giles Co., Cascades Recr. Area, 20.II.90, coll. J. Murphy, on ?*Acer*, VT 2139 (**DIK SCI fNP** TENN 56005); Washington, Kitsap Co., Bremerton, date unknown, B. McNett, on *Populus*, no. 6684 (**SPR**); Spokane Co., Spokane, 13.XI.94, coll. Kelly Chadwick, no. 6696 (**BAS-S** TENN 54828; **SPR** TENN 56054; **fNP** TENN 54828); Whatcom Co., Bellingham, 20.XI.94, coll. B. McAdoo, no 7302 (**NAT** TENN 56027; **SPR fNP** TENN 54792); Wisconsin, Waukesha Co., Kettle Moraine State Forest, X.94, coll. P. Vachuska, unknown host, V-7210 (**SPR** TENN 56002; **fNP** TENN 56041).

Culture maintenance All monokaryon and dikaryon strains were harvested from, grown and maintained on malt extract (Difco, 1.5%) agar (Difco bacto-, 2.0%; MEA). In addition, all strains are maintained as small MEA discs in microvials of sterile water (Burdsall and Dorworth, 1994).

Breeding stock Single-basidiospore isolates (SBIs) were obtained by several means: 1) from basidiomata collected in nature, basidiospores dropped on malt-agar plates following the techniques by Gordon and Petersen (1992); 2) in several instances spore prints were contributed by other workers (see acknowledgments and list of specimens and cultures used above) and these spore prints were loosened by scraping or suspension in sterile water, then diluted as described by Petersen and Hughes (1998); 3) in several instances dikaryon cultures were contributed by other workers, and these were subsequently fruited under laboratory conditions following techniques by Psurtseva and Mnoukhina (1998) and SBIs harvested as described above; 4) in the absence of all basidiospore possibilities, single-conidial isolates were used.

In several cases, after repeated subculture, SBIs became appressed to the agar surface (instead of producing copious aerial mycelium with abundant arthroconidia), often producing excessive slime, and reacted equivocally in pairing experiments. Once such a conversion took place, cultures could not be converted to their typical growth form. In *F. elastica*, this reversion necessitated a change in the exemplar from 7303 to 9004 (both Austria). These collections were gathered from the same tree a year apart, and basidiomata were morphologically conspecific. Tester strains of these collections were found to be only partially compatible (as expected from monokaryons of the same individual mycelium) and thus interchangeable. Both collections are listed above as exemplars of *F. elastica*.

Single-conidial isolates As pointed out by Aschan (1952), Brodie (1936), Ingold (1980), and Kemp (1980), dikaryon mycelia of *Flammulina* taxa produce monokaryon branches which often abstrict into arthroconidia. These arthroconidia can be diluted in various ways and germinate readily. Germlings can be harvested in the same way as germinating basidiospores, but special care

must be taken to eliminate clamp-bearing isolates from mating experiments.

Because monokaryon haploid arthroconidia are the results of mitosis and not meiosis, isolates derived from them can be useful in "recognition grids" and even perhaps in inter-exemplar pairings, where individual mating type identification is not critical, but they are useless for self-crosses, where the object is identification of all mating types represented in the collection, for only the two parental mating types will normally be expressed.

Pairing experiments Three types of pairing experiments were undertaken: 1) self-crosses, in which 12 SBIs of exemplars of each putative taxon were paired in all combinations (see Figs. 1–7, and below); 2) "recognition grids," in which four random SBIs of each "unknown" strain were paired with either: a) four SBIs of the exemplars representing each putative taxon (see Table 2); or b) with other "unidentified" strains. In these experiments, total pairings for each test equalled four (see below); and 3) inter-exemplar pairings in which 10–12 SBIs from each exemplar were paired with 10–12 SBIs of every other exemplar (Table 3). In these experiments, total pairings for each test equalled 24 (using the method reported by Petersen and Hughes, 1998).

In all cases, two small culture-bearing discs of MEA were placed about 7–9 mm apart on fresh MEA. Ample time was allowed not only for confrontation of donor mycelia between the inoculum blocks, but for contact zone differentiation to take place (usually 2–3 weeks). When examined, each pairing was scored for the following: 1) clamp connections on hyphae within the contact zone; 2) extent of lethal reactions within the contact zone; 3) presence of terminal crystalline deposits on donor mycelium to either side of the contact zone; 4) overall contact zone morphology (i.e., "flat" or other such phenomena); and 5) relative growth rates and vigor of each donor in the presence of the other.

When pairings gave equivocal or unanticipated results (i.e., scattered clamp connections restricted to the contact zone; "interspecific" hybrid dikaryons; etc.), the contact zone was excised, inverted on a fresh MEA Petri dish, and allowed to grow further. Resulting colonies were examined for clamp connections and growth vigor.

Within-exemplar self-crosses In Figs. 1–7, representing within-exemplar self-crosses, the following abbreviations appear in the grid blocks: large +=clamp connections abundant throughout the contact zone indicating a compatible pairing; small +=clamp connections occasional to common, restricted to contact zone; −=no clamp connections observed, indicating an incompatible pairing; F=sparse mycelial presence in contact zone, but not sharply defined as in typical "flat" contact zone morphology (see below under Discussion); L=significant hyphal lysis within the contact zone indicating a lethal reaction between mates; W=hyphae of one donor tightly wrapped around the hyphae of the other donor; B=a narrow transecting line of copiously branched hyphae on or in the contact zone indicating a "barrage" reaction.

In some cases, 12 SBIs were used in the initial self-cross but auxiliary SBIs were later paired with tester strains so that each mating type was represented by several monokaryon strains (i.e., *F. velutipes* var. *velutipes* no. 7200, where the mating types of 42 SBIs were identified). From each self-cross, tester strains of known mating type were selected (representing all identifiable mating types, and where necessary, supplemented by additional SBIs to produce four tester strains). A battery of such strains was deposited at CBS (Table 1).

Recognition pairings Recognition pairings were intended to provide an intersterility group name for each "unidentified" strain used in such experiments. Two methods were used to reach this end. 1) Four SBIs of each "unidentified" strain were paired with four SBIs of exemplars (n=4) of various named strains based on basidiome morphological analysis (Redhead and Petersen, 1999) and between-exemplar pairings (Table 3). This was termed a "full recognition grid." Once it was recognized that SBIs of some taxa (i.e., *F. mexicana, F. fennae, F. stratosa*, etc.) were incompatible with all SBIs outside their own taxon, the "full recognition grid" was limited to pairings of "unknowns" to exemplars of the following: *F. velutipes* vars. *velutipes, lactea* and *lupinicola, F. ononidis, F. rossica, F. elastica*, and *F. populicola*. Table 2 shows the results of these pairings. 2) Early pairing experiments paired SBIs of "unidentified" strains against SBIs of seven other "unidentified" strains (n=four). In Table 2 (summary of recognition grids), all figures are reported as numbers of compatible matches per four pairings. In cases of contamination or other anomaly, complete fractions (i.e., 1/3) represent compatible pairings over total pairings attempted. Once the "full recognition grid" experiments were completed, it was possible to assign names to more "unidentified" strains through review of these early experiments. These "unidentified strains" are not shown in Table 2, but are included by taxon name under "Collections and Cultures utilized."

Inter-exemplar pairings (usually n=24): Only SBIs whose mating types were previously assigned based on within-exemplar self-crosses were used for inter-exemplar pairings. Full complements of monokaryons (maximum=12) from each exemplar were paired with the same number of monokaryons from all other exemplars in a pattern reported by Petersen and Hughes (1998).

Table 1. Tester strains of *Flammulina* taxa deposited at CBS.

NAME/NUMBER	A_1B_1	A_2B_2	A_1B_2	A_2B_1
F. fennae (8252)	2	7	8	17
F. ononidis (CBS 172.82)	1	3	4	none
F. stratosa	14	1	2	none
F. velutipes var. *velutipes* (7200)	3	2	1	5
f. *lupinicola* (8078)	4	2	7	none
var. *lactea* (CBS 771.81)	15	2	19	12

	A_1	A_2		
F. elastica (9004)	3, 4	1, 2		
F. rossica (8171)	1, 10	7, 8		

Intercompatibility was noted as a fraction (i.e., 15/24; of 24 total pairings, 15 were compatible; see Table 3).

Results

Within-exemplar self-crosses In self-crosses of *F. velutipes* vars. *velutipes* (strain 7200; Fig. 1), *F. velutipes* var. *lactea*, (CBS 771.81) and *F. fennae* (8252; Fig 2), tetrapolar mating systems were revealed, with all four mating types identified. Similar data on *F. mexicana* have been reported elsewhere (Redhead, et al., 1999a). In *F. velutipes* var. *lupinicola* (8078; Fig. 3), *F. ononidis* (CBS 172.82; Fig. 4), and *F. populicola* (DED 6115; Fig. 5), tetrapolar mating systems were revealed, but only three mating types could be identified. Similar self-cross data were reported elsewhere for *F. stratosa* (Redhead, et al., 1999b). Even in those cases where four mating types could be assigned to isolates, individual numbers of mating types were unbalanced (i.e., instead of three isolates of each of four mating types, some mating types were more numerous than others). This was obvious in those self-crosses where only three mating types were assignable. In self-crosses of *F. rossica* (8171; Fig. 6) and *F. elastica* (9004; Fig. 7) only two mating types were identifiable, indicating a bipolar mating system, but other factors may indicate a tetrapolar mating system with very unbalanced numbers of assignable mating types (see Discussion below).

Recognition pairings It was established in inter-exem-

Fig. 2. Self-cross grid for *Flammulina fennae* (strain 8252; ex exemplar). Large + =abundant clamp connections throughout the contact zone; small + =scattered clamp connections restricted to contact zone; − =no clamp connections observed; B=ill-defined "barrage" contact zone morphology; L=significant hyphal lysis in contact zone.

• 6 = $A_1B_1 + A_2B_1$; 7 = $A_2B_2 + A_1B_2$

Fig. 1. Self-cross grid for *Flammulina velutipes* (no. 7200; ex epitypus). + =abundant clamp connections throughout the contact zone; − =no clamp connections observed; F=ill-defined "flat" contact zone morphology (see text for explanation).

• $A_2B_1 + A_2B_2$
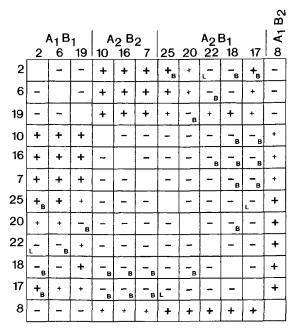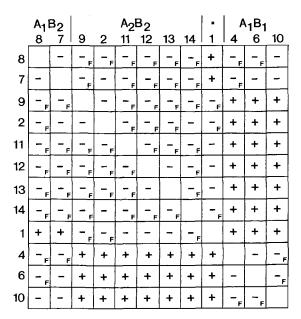
Fig. 3. Self-cross grid for *Flammulina velutipes* var. *lupinicola* (strain 8078; ex holotypus). + =abundant clamp connections throughout the contact zone; − =no clamp connections observed; F=ill-defined "flat" contact zone morphology.

Table 2.   Mating groups in *Flammulina*.

NAME/STRAIN *F. velutipes*	*F. velutipes* (7200)	var. *lupinicola* (8078)	var. *lactea* (CBS 771.81)	*F. ononidis* (CBS 172.82)	*F. rossica* (8171)	*F. elastica* (7303)	*F. populicola* (7271)	*F. fennae* (8252)
2359		3	4	0	0	0	0	0
2413		4	4	4	2	0	0	
4196		3/3	3/3	1/3	0	0	0	
4400		3	3/3	0	0	0	0	
4631		3	4	3	2	0	0	
6201		4	2	3	1	0	0	
62011		4	3	3/3	0	0	0	
6694		4	4	3/3	0	0	0	
6696		4	4	3/3	2	0	0	
6698		1	4	3	1	0	0	
7199		4	4	3/3	0	0	0	
7215		2	4	4	0	0	0	
7301		4	4	3/3	2	0	0	
73011		4	4	4	1	2	0	
7305		3	4	4	2	1	0	
7308		4	4	3/3	1	1	0	
7310		4	4	3/3	1	0	0	
7318		4	4	4	0	0	0	
7321		2	4	2/3	0	0	0	
7322		4	4	4	0	0	0	
7368		4	4	4	0	0	0	
8015		4	4	2	3	0	0	
8024		4	3/3	4	1	0	0	0
8316		3/3	3/3	0/2	0/3	0/1	0	0/1
8326		4	4	4	0	1	0	0
8359		3	4	1	0	0	0	0
8391		3	4	4	1	1	1/3	
8952		4	4	0	4	0	0	0
9005		4	4	3/3	1	0	0	
9061		4	4	3/3	0	0	0	
ASM 5025		4	3/3	3	0	1	0	0
ASM 8022		3/3	3/3	2	0	0	0	0
DAOM 1294		0	3	0	0	0	0	0
DAOM F-1598	2	1/3	0	0	0	0	0	
DAOM 17788		4	4	4	2	1	0	
DAOM 188690	4	4	4	1	1	0		
DAOM 197533	3	3	3	2	0	0		
HBB 13560		4	4	3/3	2	0	0	
IFO 30905		4	4	4	0	0	0	
JRB 367	1/3	3/4	0	3	0	0		
LE 671		4	4	4	3	2	0	
LE 817		3	4	3	0	0	0	
LE 905		4	4	2	1	2	0	
LE P		3	4	4	0	0	0	
LE T		4	4	4	0	0	0	
MUCL 28497		4	4	4	0	0	0	
MUCL 31627		4	4	4	1	0	0/3	

Table 2. Continued.

NAME/STRAIN F. velutipes	F. velutipes (7200)	var. lupinicola (8078)	var. lactea (CBS 771.81)	F. ononidis (CBS 172.82)	F. rossica (8171)	F. elastica (7303)	F. populicola (7271)	F. fennae (8252)
MUCL 38390	4	4	4	1	0	0/3	0	
NZFR 243D	4	4	4	0	0	0	0	
THON 293	4	4	3	1	1	0	0	
VT 0058	4	2	4	1	0	0	0	
VT 1127	4	4	4	2	1	0	0	
VT 1576	3	4	3	0	0	0	0	
VT 2015	4	4	3/3	0	0	0	0	
VT 2139	4	4	4	2	1	0	0	
ROSSICA								
3232	0	0	0	0	4	3/3	0	0
BULLAKH	0	0	0	0	4	3/3	0	0
LE 388	0	0	0	0	4	4	0	0
LE 389	0	0	0	0	4	3/3	0	
LE 535	0	0	0	0	4	3/3	0	0
LE 904	0	1/3	0	0	4	4	0	
LE N	0	0	0	1	4	3/3	0	
MAGADAN	0	0	0	0	4	3	0	
ELASTICA								
9002	0	0	0	0	0	4	0	
POPULICOLA								
4244	0	0	0	0	0	0	4	0
6685	0	0	0	1	0	0	3	0
6704	0	0	0	1	0	0	3	0
7278	0	1	0	1	0	0	4	0
8196	0	1	0	0	0	0	4	0
8200	1/3	0	0	0	0	0	2	
8202	1	0	0	0	0	0	4	
8203	0	1	0	0	0	0	0	
DED 6115	1	2	0	1	0	0	4	0
FVC 1	2	0	0	2	0	0	4	
GRUEN	0	0	0	1	0	0	4	0
GRUEN 3-6	1	2	1	2	0	0	4	0
HALLING 7495	2	1	0	0	0	0	4	
IFO 30490	1	1	0/3	0	0	0	4	
SPEARMAN 32	1	0	0	1	0	0	2	0
MEXICANA								
5237	0	0	0	0	1	0	0	0
PANCOMPATIBLE STRAINS								
OLEXIA 2	4	4	2	0	4	4	0	0
IFO 30875	4	4	2	0	4	4	0	0

plar experiments (see below) that SBIs of *F. fennae* (strain 8252), *F. stratosa* (strain 7099), and *F. mexicana* (strain 6237) were incompatible with those of all other strains and thenceforth were dropped from subsequent recognition grid experiments. Taxa remaining in the recognition grids were *F. velutipes* var. *velutipes*, var.

lactea and var. *lupinicola*, *F. ononidis*, F. populicola, *F. rossica*, and *F. elastica*. Table 2 presents results of recognition grid experiments. Taxa are arranged in alpha-numerical order within groups of putative taxa.

Two categories of results were distinguished: 1) unidentified strains could be grouped into those compati-
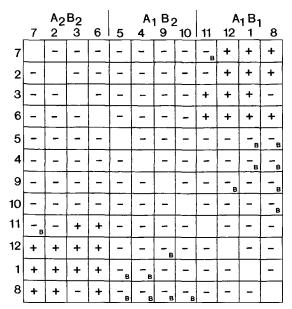
Fig. 4. Self-cross grid for *Flammulina ononidis*.

	A2B2				A1B2				A1B1			
	7	2	3	6	5	4	9	10	11	12	1	8
7		−	−	−	−	−	−	−	−B	+	+	+
2	−		−	−	−	−	−	−	−	+	+	+
3	−	−		−	−	−	−	−	+	+	+	−
6	−	−	−		−	−	−	−	+	+	+	+
5	−	−	−	−		−	−	−	−	−	−B	−B
4	−	−	−	−	−		−	−	−	−	−B	−B
9	−	−	−	−	−	−		−	−	−	−B	−B
10	−	−	−	−	−	−	−		−	−	−	−B
11	−B	−	+	+	−	−	−	−		−	−	−
12	+	+	+	+	−	−	−B	−	−		−	−
1	+	+	+	+	−B	−B	−	−	−	−		−
8	+	+	−	+	−B	−B	−B	−B	−	−	−	

Fig. 4. Self-cross grid for *Flammulina ononidis*. (monokaryon isolates from basidioma from CBS 172.82). + =abundant clamp connections throughout the contact zone; − =no clamp connections observed; B=ill-defined "barrage" contact zone morphology.
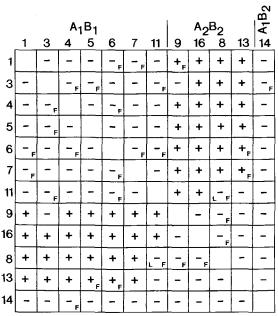
Fig. 6. Self-cross grid for *Flammulina rossica* (strain 8171).

	A1						A2			
	1	5	9	10	13	15	2	4	8	7
1		−	−	−F	−	+	+	+	+	−F
5	−		−F	−F	−F	+	+	+	+	−F
9	−	−F		−	−	x	+	+	+	+
10	−F	−F	−		−F	+	+	+	+	−F
13	−	−F	−	−F		x	+	+	+	+
15	+F	+	x	+	x		−F	x	x	−
2	+	+	+	+	+	−F		−	−	−B
4	+	+	+	+	+	x	−		x	−
8	+	+	+	+	+	x	−	x		−
7	−F	−F	+	−F	+	−F	−B	−	−	

Fig. 6. Self-cross grid for *Flammulina rossica* (strain 8171, ex exemplar). + =abundant clamp connections throughout the contact zone; − =no clamp connections observed; F=ill-defined "flat" contact zone morphology; B=ill-defined "barrage" contact zone morphology; X=inoperative pairing.

Fig. 5. Self-cross grid for *Flammulina populicola* (strain 7271).

	A1B1							A2B2				A1B2
	1	3	4	5	6	7	11	9	16	8	13	14
1		−	−	−	−F	−F	−	+F	+	+	+	−
3	−		−F	−F	−	−	−F	−	+	+	+	−F
4	−	−F		−	−F	−	−	+	+	+	+	−
5	−	−F	−		−	−	−	+	+	+	+	−
6	−F	−	−F	−		−F	−F	+	+	+	+F	−
7	−F	−	−	−F			−	+	+	+	+F	−
11	−	−F	−	−	−F	−		+	+	−LF	−	−
9	+	−	+	+	+	+	+		−	−F	−	−
16	+	+	+	+	+	+	+	−		−F	−	−
8	+	+	+	+	+	+	+	−LF	−F		−	−
13	+	+	+	+F	+F	+	+	−	−	−		−
14	−	−	−F	−	−	−	−	−	−	−	−	

Fig. 5. Self-cross grid for *Flammulina populicola* (strain 7271; ex exemplar). + =abundant clamp connections throughout the contact zone; − =no clamp connections observed; F=ill-defined "flat" contact zone morphology; L=significant hyphal lysis within contact zone.

Fig. 7. Self-cross grid for *Flammulina elastica* (strain 9004).

	A1			A2								
	19	2	3	4	5	6	7	9	10	12	1	20
19		−F	−F	+	+	+	+	+	+	+	+	+
2	−F		−F	+	+	+	+	+	+	+	+	+
3	−F	−F		+	+	+	+	+	+	+	+	+
4	+	+	+		−B	−F	−B	−	−	−	−	−
5	+	+	+	−B		−FL	−F	−B	−F	−FL	−F	−F
6	+	+	+	−F	−F		−	−L	−F	−	−LF	−F
7	+	+	+	−BL	−F	−		−LF	−B	−BL	−	−F
9	+	+	+	−B	−LFL	−F	−		−	−LF	−L	−L
10	+	+	+	−	−F	−B	−		−	−	−	−F
12	+	+	+	−	−F	−	−BL	−F		−B	−	−
1	+	+	+	−	−LFL	−FL	−	−	−B		−	−
20	+	+	+	−	−F	−F	−L	−	−F	−		

Fig. 7. Self-cross grid for *Flammulina elastica* (strain 9004, ex exemplar). large + =abundant clamp connections throughout the contact zone; small + =scattered clamp connections limited to contact zone; − =no clamp connections observed; F=ill-defined "flat" contact zone morphology; B=ill-defined "barrage" contact zone morphology; L=significant hyphal lysis in contact zone.

ble with: a) the infraspecific taxa of *F. velutipes* (i.e., its varieties) and to a lesser extent, *F. ononidis* ; b) *F. elastica* and/or *F. rossica*; c) *F. populicola*; and 2) scattered pairings outside these groupings [i.e., *F. velutipes* ×
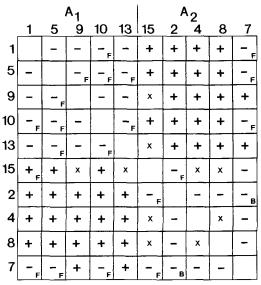
F. populicola (8391×7271); *F. velutipes*×*F. rossica* (7308×8171)].

Inter-exemplar pairings Table 3 shows the results of inter-exemplar pairings. SBIs of *F. fennae* (strain 8252),

Table 3. Inter-exemplar pairing compatibility.

	F. velutipes 7200	var. lupinicola 8078	var. lactea 771.81	F. ononidis 172.82	F. populicola 7271	F. rossica 8171	F. elastica 9004*	F. fennae 8252
		NAME AND ISOLATE NUMBER						
7200		24/24	21/23	16/24	2/24	0/19	2/22	0/24
8078			24/24	24/24	0/22	2/23	0/24	0/24
771.81				15/24	0/22	0/24	0/22	0/24
172.82					0/24	0/20	0/24	0/24
7271						0/24	0/24	0/24
8171							9/22*	0/24
9004								0/24
8252								

· 7303 × 8171 = 15/23

F. mexicana, and *F. stratosa* were incompatible with those of each other as well as with those of all other exemplars. By implication, these four taxa are genetically isolated, and for *F. stratosa* and *F. mexicana* these data have been reported elsewhere (Redhead et al., 1999a, 1999b).

In separate experiments, SBIs of exemplars of *F. elastica* (7303 and 9004 from Austria) were proven to be totally interchangeable. Because morphological analyses of basidiomes of these collections led to the same conclusion, SBIs of 9004 were substituted for those of 7303 which had deteriorated over time. Nonetheless, SBIs of both exemplars were used in pairing experiments with *F. rossica*. In both experiments, partial intercompatibility was observed (7303 × 8171 = 9/22; 9004 × 8171 = 15/23). Such results suggest that gene flow between populations of these taxa might be possible.

Inter-exemplar pairing experiments among the infraspecific taxa under *F. velutipes*, and their behavior in recognition grids (Table 2), showed them to be virtually totally intercompatible (Table 3). Pairing experiments between the exemplar of *F. ononidis* and exemplars of the infraspecific taxa within *F. velutipes* revealed total compatibility (i.e., *F. ononidis* × *F. velutipes* var. *lupinicola*) to partial compatibility (i.e., *F. ononidis* × *F. velutipes* var. *velutipes*; *F. ononidis* × *F. velutipes* var. *lactea*).

Culture micromorphology Two unusual categories of hyphal differentiation were encountered. First, as reported by Aschan (1952), Brodie (1936) and Ingold (1980), dikaryon cultures of *F. velutipes* produce monokaryon branches which abstrict monokaryon arthroconidia. Our study, however, shows that Aschan was working with *F. populicola*, not *F. velutipes*. Our observations, therefore, confirm that this dedikaryotization phenomenon also extends to *F. populicola*. *Flammulina rossica*, conversely, produced dikaryon arthroconidia (i.e., binucleate under epifluorescence microscopy; germlings binucleate and clamped). Thus, Aschan's (1952) observations seem not to extend to the entire genus.

Second, in seemingly random pairings (but most often involving *F. rossica*), small, undifferentiated hyphal side branches were observed, surmounted with a small, hyaline, liquid (i.e., clinging to a fine needle) droplet. In time, these droplets appeared to slowly disappear, but in their place a cluster of extremely fine (i.e., <0.3 µm broad) crystals appeared, at first hyaline, but later becoming pale tan to straw-colored. These crystals extruded to variable lengths, but often over 15 µm. In mass, these crystalline deposits gave cultures of *F. rossica* (and less commonly other taxa) a pallid tan color. Ingold (1980) termed these hyphal tips as "aspergilloid hyphae," but he found the structures on basidiome initials and on hyphal ganglia which he construed to be potential basidiome sites. We found no such specialization, with the "aspergilloid hyphae" randomly scattered, but most common in or near the contact zone of certain pairings, usually interspecific and usually incompatible. *Flammulina rossica* was particularly prone to produce these structures.

In addition, certain SBIs formed plate-like crystals ranging from blue to purple. Occasionally, such crystals formed in macroscopic sheets, but such phenomena were sporadic and seemingly without pattern.

Discussion

Within-exemplar self-crosses Previous reports of tetrapolarity (Brodie, 1936; Lamoure, 1989; Vandendries, 1937) not withstanding, virtually no self-cross in our experiments, whether illustrated here or not, yielded unequivocal results. The following factors require separate exposition: 1) interpretation of contact zone morphology; 2) unbalanced ratios of mating types; 3) alternative interpretations of self-cross grids; and 4) amphithallic behavior.

In *Flammulina* pairings (including self-crosses), contact zone morphology was not easily interpreted. From our experience (primarily RHP), typical "flat" contact

zone morphology, perhaps best exhibited in *Schizophyl-
lum commune*, various polypores, and *Lentinus* taxa, in-
cludes a well-defined "crevasse" between donor colo-
nies, often accompanied by significant hyphal lysis, and
sometimes bounded by zones of congested hyphal
branching by donor colonies. "Flat" as annotated on
Flammulina self-cross grids comprised a wide (4–8 mm)
area of sparse mycelium, rarely accompanied by hyphal
lysis, and almost always bounded by somewhat congest-
ed hyphae. Equally important, the *Flammulina*-type
"flat" was unpatterned, not reflective of mating type
control. Figures 1–7 all show the *Flammulina*-type
"flat," and in all cases it was unpatterned. Hyphal lysis
is also shown in these figures, often unrelated to "flat."

The typical "barrage" contact zone morphology,
again exhibited by the organisms mentioned above,
comprises a relatively well-defined stripe of congested
mycelium which, if found on di-di or di-mon pairings
("vegetative incompatibility") would be termed a "non-
self" reaction. In some organisms, this morphology can
be accompanied by production of false clamp connec-
tions. In *Flammulina*, "barrage" was ill-defined, always
aerial, and never accompanied by false clamp connec-
tions. Like "flat," "barrage" was never found to be pat-
terned (see Figs. 2, 4, 7).

Yet another phenomenon which renders contact
zone morphology almost useless in self-cross grid inter-
pretation is within-mating type differentiation. In Figs. 1
and 3, for example, "flat" contact zone morphology is
common in pairings within the same mating type, and in
Fig. 7. "barrage" can be identified also in within-mating
type pairings.

Because contact zone morphology is of little help in
self-cross grid interpretation, subordinate mating types
(A_1B_2; A_2B_1) were always assigned arbitrarily.

In many self-crosses, the ratio of mating types was
skewed. In Figs. 1 and 2, this ratio approaches the ideal
of 3 : 3 : 3 : 3 (when 12 SBIs are employed). The level of
skewing shown in Figs. 1 and 2 was clearly within tolera-
ble limits. Figures 3–5, however, show a more severe
level of skewing, where only three mating types were
assignable. Nonetheless, such results are common in
Hymenomycete self-crosses and so are not unexpected.
Figure 5 (*F. populicola*) shows even more severe skew-
ing, with only a single isolate representing the third mat-
ing type, and no representation of the fourth. Were this
single monokaryon isolate replaced by another represent-
ing A_1B_1 or A_2B_2, the grid would superficially appear as
bipolar, not tetrapolar.

This situation, carried to one more level of extreme
skewing, could produce grids like those shown in Figs. 6
and 7, where seemingly only two mating types appear.
In Fig. 7 (*F. elastica*), the only indication of the usual sus-
pected tetrapolar behavior is the presence of *Flammulina*-
type "flat" contact zone morphologies. But (see above)
such morphologies are unlinked to mating type control,
and this, together with a significant number of inopera-
tional pairings (i.e., stunted growth of one or both donors;
contamination; etc.) makes definitive interpretation of
this grid very speculative.

Likewise, an alternative explanation of the grid in
Fig. 6 can be offered. The following interpretive summa-
ry also corresponds to the grid: isolates 2, 4, $8=A_1B_1$; 7
$=A_2B_1$; $15=A_1B_2$; 9, $13=A_2B_2+A_1B_2$; 1, 5, $10=A_2B_2$
$+A_2B_1$; A_2B_2 is missing. Such an interpretation clearly
requires tetrapolarity.

We are not prepared, therefore, to define *F. rossica*
and *F. elastica* as bipolar members of an otherwise tetra-
polar genus. That these two morphotaxa also happen to
form a genetic complex (i.e., partially intercompatible, but
almost totally interincompatible with other taxa) may be
coincidental, may indicate a shared severe skewing of
mating type frequency, or suggest that they are bipolar.
Low-level compatibility between *F. elastica/rossica* and
other tetrapolar exemplars (Table 3) supports the conclu-
sion that self-crosses of *F. elastica* and *F. rossica* are
exhibiting highly skewed tetrapolarity. Obviously, more
work is necessary in this complex.

Figures 1 and 3 (and Fig. 6 if the above alternative
interpretation is accepted) show some instances of dual
compatibility. This could be caused by occasional un-
directed amphithallism (Petersen, 1995). The level of
amphithallism seems low, unlike that in the Stropharia-
ceae (McCleneghan, 1996 for *Pholiota*; Rehner, 1989 for
Agrocybe; etc.)

Recognition grids As stated above, two categories of
results can be seen in Table 2. First, monokaryon iso-
lates from "unidentified" collections were chiefly com-
patible with one of the following groups of putative taxa:
1) the infraspecific taxa within *F. velutipes* and less so
with *F. ononidis*; 2) *F. elastica/F. rossica*; and 3) *F.
populicola*.

The most complex interbreeding pattern was ob-
served among the infraspecific taxa under *F. velutipes*
together with the anomalous *F. ononidis*. Redhead and
Petersen (1999) described *F. velutipes* var. *lupinicola*
from a limited habitat on *Lupinus arboreus* in California on
the North American Pacific coast. Varieties *velutipes*
and *lupinicola* are virtually completely intercompatible
(Tables 2, 3). Basidiospore statistics separate var.
velutipes and var. *lupinicola*, the latter of which seems
limited to the salty windswept coastline. *Flammulina
velutipes* var. *lactea* seems merely to be an albino or
semi-albino morph of *F. velutipes*, occurring uncommon-
ly throughout the range of *F. velutipes*. It is no surprise,
therefore, that var. *lactea* and var. *velutipes* are virtually
completely intercompatible. *Flammulina ononidis*, how-
ever, was described from Germany as occurring on the
roots of *Ononis*, a genus of the Fabaceae. To our knowl-
edge, the only cultures of *F. ononidis* were at CBS, and a
dikaryon culture was fruited in our laboratory to yield
monokaryon isolates. Although morphological analysis
(Arnolds, 1977) had not indicated a close relationship
to *F. velutipes*, recognition grids (Table 2) and inter-
exemplar pairings (Table 3) consistently revealed partial
compatibility between *F. ononidis* and all infraspecific
taxa within *F. velutipes*. Such results indicate a closer
genetic relationship than heretofore reported. This
strain, however, did not produce the characteristically
large basidiospores of the species when fruited in the lab-

oratory.

Without seeking out the specific strains used in previous reports on *Flammulina* development, physiology, and commercial strain improvement, it is difficult to assess the results of previous studies, especially as they deal with fruiting. Examples may suffice. Gruen generously contributed two dikaryon strains of *F. "velutipes"* used in several of his reports (Gruen, 1969, 1976, 1979, 1982, 1983; Wong and Gruen, 1977; Gruen and Wu, 1972). Once fruited for basidiome morphological analysis (Redhead and Petersen, 1999), and once SBIs from those fruitings were submitted to a "recognition grid," it was clear that both strains represented what Redhead and Petersen (1999) have called *F. populicola*, a taxon almost completely interincompatible with *F. velutipes*. Not only does this mean that Gruen's data do not apply to *F. velutipes*, but McKnight (1990, 1992) and McKnight and Estabrook (1986) used Gruen's strain "3-6," originally obtained from Aschan (1952; Aschan-Åberg, 1958) from Sweden, in concluding that strains of *F. "velutipes"* differed in their ability to fruit at varying relative humidities. Conclusions based on those data must also be reviewed. Furthermore, Yokoyama (1991) used strain IFO 7777 in his mating study, but IFO 7777 was obtained from Gruen, was isolated from nature in Saskatchewan, Canada, and is a duplicate of what is herein called "Gruen." As such, IFO 7777 also represents *F. populicola*. Yokoyama's (1991) conclusion that two intersterility groups existed within *F. "velutipes"* was correct, although we consider these groups as discrete species. In fact, if Yokoyama's distribution maps are superimposed on ours, his results presage ours. We cannot conclude, however, what taxon might have been involved in the research by, for example, Plunkett (1953, 1956; coming from United Kingdom; it probably was *F. velutipes*) or Takemaru (1954–1961, Takemaru et al., 1995, etc.; coming from Japan; it probably was *F. velutipes*).

There are difficulties in interpreting prior reports of pale or white pilei. *Flammulina velutipes* produces such basidiomata, SBIs of which are completely intercompatible with those derived from basidiomata with typical orange-brown to russet pilei. Thus *F. velutipes* var. *lactea* cannot be separated from var. *velutipes* by mating tests alone. Conversely, basidiomata of *F. fennae* and *F. rossica* were described with cream to white pilei. SBIs of both taxa are universally interincompatible with those of each other, and virtually so with those of the *F. velutipes* complex. Such observations obfuscate such reports as that by Kirby and Sleath (1995) on color variation in *F. velutipes*.

Redhead and Petersen (1999) concluded that some reports of a long-spored *F. velutipes* could be referred to a separate species based on pileipellis characters. In western Europe it often fruits on *Salix* spp. At species rank, *F. elastica* (Lasch) Redh. and Pet. appears to be the correct binomial (Redhead and Petersen, 1999). SBIs of several collections of this species were intercompatible, but nearly universally interincompatible with those of the *F. velutipes* complex. Lamoure's (1989) report of partial

compatibility between *F. velutipes* f. *velutipes* and f. *longispora* (in most cases "*ad salicem*") are less easily interpreted but do not contradict our observations. The report of incompatibility between SBIs of *F. velutipes* "f. *longispora*" (from "deciduous tree") and those of *F. ononidis* (Klán et al., 1992) reflect their taxonomic separation.

In mating experiments, we used one collection with long spores combined with pileipellis structure typical of *F. velutipes* from North America. It appears in Table 2 as "Olexia," and was compatible with several morphological taxa. It may represent a "pancompatible" strain much like the *Pleurotus* strain reported by Petersen and Ridley (1996).

Likewise, the report by Vellinga (1996) on distribution of *Flammulina* in Europe must be interpreted against our observations. Two collections generously contributed to our study by Dr. Vellinga under the name *F. velutipes* f. *longispora* and collected on *Salix*, represent what we consider to be *F. elastica*.

Morphologically *F. elastica* (pileipellis an ixotrichodermium) differs significantly from *F. rossica* (pileipellis an ixohymenidermium or ixosubhymenidermium; Redhead and Petersen, 1999) but SBIs of the two are partially compatible in the neighborhood of 50%. The two are clearly more closely related sexually than their morphology would suggest. The geographic range of *F. elastica* seems limited to western Europe and Finland, while the range for *F. rossica* covers Europe, northern Asia, and the Pacific coastal regions of North America.

Putative cultures of *F. fennae* from CBS were anomolous within the genus. Specifically, as reported by Bas (1983): 1) basidiospores germinated slowly (a matter of weeks) and in extremely low numbers, both atypical of the genus; and 2) when paired with isolates of *F. "velutipes,"* isolates of *F. fennae* not only dominated (not unusual for certain pairings within the genus), but devoured their mates (this could be an interpretation of "lethal" contact zone phenomena common in pairings of many agarics). In addition, we have observed that: 1) mono- and dikaryon isolates slowly produced a golden yellow trichodermium of elongate-clavate, lightly crystal-incrusted hyphal tips, unique in this genus; 2) no arthroconidia were produced, again unique for this genus; 3) RFLP analyses show these CBS cultures to be basal and relatively unrelated to all other cultures in the genus (Methven et al., 1997, 1999); and 4) DNA sequences derived from paratype dried basidiomata of *F. fennae* (J. Johnson, pers. comm.) did not resemble those of CBS cultures, but were virtually identical to those derived from 8252 (Hughes, et al., 1999), a second collection of morphospecies *F. fennae* (see list of specimens and cultures used, and below). We conclude that the CBS cultures do not represent a *Flammulina*. Hence, data from previous publications which included those cultures as exemplars of *F. fennae* must be dismissed.

One collection of *F. fennae*, 8252, represented by dried basidiomata from nature, a dikaryon culture derived from one of those basidiomata, laboratory-fruited basidiomata and SBIs derived from them, was uniquely

interincompatible with all other collections of all other taxa. Basidiome morphology closely resembled that of *F. fennae* (Bas, 1983), but cultures (both dikaryon and SBIs) were white, lannose to loosely cottony, with abundant aerial mycelium and copious arthrospores, all different from CBS cultures of *F. fennae*. DNA sequences derived from cultures of 8252 were typical of *Flammulina*, as were colony growth rate, pattern and micromorphology. These sequences also matched DNA sequences derived from a paratype specimen of *F. fennae* (J. Johnson, pers. comm.). For these reasons, cultures of 8252 were ultimately adopted as a substitute exemplar to represent *F. fennae*.

It would appear that putative interspecific hybridization can occur via two routes. In one, certain collections were found to be compatible with several other taxa (i.e., Table 2; "Olexia" and IFO 30875), much as reported by Petersen and Ridley (1996) for New Zealand *Pleurotus pulmonarius*. If these collections represent a phenomenon occurring in nature, gene flow could take place between different morphological taxa through these pancompatible individuals.

A second category of results included in Table 2 deals with compatible pairings between SBIs of taxa not expected to show such results (i.e., under *F. populicola*, occasional compatibility with SBIs of *F. velutipes*). Not only were these pairings judged compatible by observation of clamp connections in the contact zone, but when contact zones were excised and allowed to proliferate into colonies, resultant colonies were characterized by presence of abundant clamp connections. There seems to be no adequate conclusion other than that these occasional anomalous compatible pairings produce proliferating hybrid dikaryons. In three instances, such hybrid dikaryons were fruited and produced more or less normal basidiomata with viable basidiospores (unpubl. data). Several additional hybrid dikaryons have been maintained for subsequent fruiting experiments and analysis of whether such characters as basidiospore statistics, pileipellis structure and hyphal details represent intermediates between the parental strains or whether certain characters are inherited on a dominant/recessive basis. Redhead (unpubl. data) has identified many basidiomata from nature and from fruited strains which seem to represent morphological intermediates between taxa according to pileipellis structure and spore dimensions, so hybridization events reported here should not be a total surprise.

Additional data can be inferred from Table 2. Names in left headings were largely derived from mating data, but basidiomata were often also examined by SAR and annotated for morphological characters. With this in mind, collections appearing under *F. velutipes* were virtually universally incompatible with those of *F. populicola* (column 7). Conversely, those collections judged by mating tests to be *F. populicola* were significantly more likely to form dikaryons with *F. velutipes* (all infraspecific variants).

Concommitantly, if collections under *F. populicola*, noted for their sporadic compatibility with *F. velutipes*,

are compared with collections of *F. elastica* and/or *F. rossica* (columns 5 and 6; with partially compatible gene pools), it is found that the *F. elastica/rossica* intersterility group almost nevers dikaryotizes *F. velutipes* (including all infraspecific variants). It might be speculated from mating experiments, therefore, that *F. populicola* is "closer" (i.e., shows greater ability to dikaryotize) to *F. velutipes* than is the *F. elastica/rossica* complex. Conversely, DNA sequences place these taxa at opposite ends of a phylogeny (Hughes et al., 1999).

Tables 2 and 3 show that collections (including exemplars) of *F. elastica/rossica* and *F. populicola* are totally interincompatible. Again, it could be speculated that these two complexes are genetically isolated from one another. Thus, of these three complexes (i.e., *F. velutipes*, *F. elastica/rossica*, *F. populicola*), *F. populicola* is "closer" to *F. velutipes*, but *F. elastica/rossica* is almost genetically isolated from both.

Such low-percentage "interspecific" hybridization events are not limited to *Flammulina*. Petersen and Hughes (1998) showed similar results in *Omphalotus*, Vilgalys and Miller (1987a, b) reported such figures within the European *Collybia dryophila* group, Johnson (1997) found this situation in the *Xeromphalina campanella* complex, and Scott Gordon (unpubl. data) has observed it in sect. *Androsacei* of *Marasmius*. There is little doubt that "interspecific" hybridization will be reported more frequently as appropriate studies are undertaken in other groups. Thus, probable interspecific hybridization can be added to the list of "novelties" occurring in the agarics (see above under Introduction).

In cases where micromorphological analysis of basidiomata from nature were supplemented by those on basidiomata of the same strain fruited in the laboratory, significant variation was often observed, especially in spore length measurements and often in pileipellis details (Redhead, pers. comm.). With laboratory conditions varying much less than those under which fruiting occurs in nature, it might be hoped that basidiomata fruited *in vitro* might duplicate their *in vivo* parent basidiomata, indicating stability of characters. Instead, *in vitro* basidiomata usually differed from *in vivo* basidiomata in pileipellis micromorphology and spore statistics, as well as some expected macroscopic characters such as basidiome color, stature, and flesh thickness. From these data, the distinction between genetic variation and phenotypic plasticity is rendered less clearcut. To be sure, mating experiments largely confirm identifications by morphological means, as do RFLP patterns and DNA sequences (Methven et al., 1997, 1999; Hughes et al., 1999).

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