

INTERCONTINENTAL COMPATIBILITY IN *PANELLUS STYPTICUS*
WITH A NOTE ON BIOLUMINESCENCE

RONALD H. PETERSEN

*Knoxville**

DAVID BERMUDEZ

*Milwaukee***

Single-spore and polypore cultures of *Panellus stypticus* from Japan were non-luminescent, while single-spore isolates from Illinois comprised both luminescent and non-luminescent strains. Luminous single-spore isolates exhibited varying levels of luminescence, and in some single-spore isolates, a delay in the onset of luminescence was observed. Japanese and eastern North American collections were universally sexually compatible, and thus belong to the same biological species. Nuclear migration beyond the contact zone between crossed single-spore strains was only occasionally observed, and was consistently accompanied by hyphal lysis of the receptor strain. Production of nuclei fit for nuclear migration appeared strain-specific, and not related to mating type genes.

It was reported previously (Macrae, 1937, 1942) that *Panellus stypticus* (Bull.: Fr.) Karst. from eastern North America exhibited a bifactorial (tetrapolar) mating system (confirmed by Burdsall & Miller, 1971), and was sexually compatible with the same morphospecies from Europe. To this time, no data on compatibility of Asian strains with North American isolates have been furnished.

Macrae also stated that North American material of *P. stypticus* exhibited bioluminescence, while 'Eurasian' collections were without this biochemical phenomenon. Her observations, however, were made by naked eye, not enhanced by photometric methods, and there is no evidence that she used Asian strains in her analysis.

In 1989, single-spore and polypore cultures of *P. stypticus* were established from central Japan, and from Illinois and Tennessee in North America. The purpose of this study was to ascertain whether *P. stypticus* from Japan was compatible with the same morphospecies from eastern North America, and to ascertain more accurately bioluminescence of strains from both continents.

MATERIALS AND METHODS

Collections. — Cultures (single-spore assumed monokaryon, and polypore assumed dikaryon) were established from the following collections: JAPAN. Gunma Pref., Lake Kusake, Tokyo University of Agriculture and Technology preserve, 26.IX.1989, RHP 2300

* Botany Department, University of Tennessee, Knoxville, Tennessee 37916, U.S.A.

** Center for Great Lakes Studies, University of Wisconsin, Milwaukee, Wisconsin 53204, U.S.A.

(TENN 48156); Tochigi Pref., vic. Lake Chuzenjiko, on ? *Chaemaecyparus* log, 27.IX. 1989, *RHP* 2315 (TENN 48762). UNITED STATES. Illinois, Jackson Co., Touch of Nature Reserve, 20.X.1989, coll. by North American Mycological Association members, *RHP* 2416 (TENN 48763); Tennessee, Sevier Co., Great Smoky Mountains National Park, Bullhead Trailhead, on hardwood log, 19.I.1990, *RHP* 2536 (TENN 48764).

Cultures. — Pilei or portions of pilei including several lamellae were suspended from the lid of a Petri dish with petroleum jelly, and spores were shed onto malt extract agar (MEA; 1.5% Difco malt extract, 2.0% Difco bacto-agar, in distilled water). After spore germination, single-spore germings were transferred to another identical Petri dish and allowed to grow. When colonies increased to c. 10 mm diam., three subcultures were made from each single-spore colony: (1) an agar block from each colony was placed in sterile 10% glycerol in a small vial, and stored at -70°C for long-term inoculum; (2) an agar block was transferred to a slanted MEA culture tube and stored at 4°C for short-term inoculum; and (3) an agar block was transferred to a small Petri dish of MEA for growth as inoculum for mating.

Crosses were made on MEA in small (60 × 15 mm) Petri dishes by placing small inoculum blocks (c. 3 mm square) about 10 mm apart. Resultant colonies overgrew between the inoculum blocks, establishing a contact zone.

Interpretation of matings. — Contact was made between mated isolates in about three days, but crosses were not read until at least two weeks after mating, so that possible contact zone morphological differentiation could be observed, as well as nuclear migration.

Crosses were judged compatible by presence of common clamp-connections on hyphae in the contact zone. These common clamps were readily observed at 400 × on hyphae at the agar surface without resort to higher magnification. Often, only scattered to rare clamp-connections could be observed, and these crosses were interpreted as including common B mating type genes (Raper, 1966). It was necessary in such cases to examine the clamps more closely to ascertain whether they were 'false clamps' (not fused to the parent hypha), and this was accomplished by placing a small shard of cover glass on the agar surface and observing the structure at 800 ×.

In all intercollection matings, areas of mated colonies away from the contact zone were searched for clamp-connections. Such clamp-connections, when found, were judged to be indicative of nuclear migration away from the contact zone.

Tester strains. — For each collection, four tester strains were selected on two criteria: (1) each mating type was represented; and (2) abundance of clamp-connections in each cross was judged. In collection *RHP* 2300, tester strains were selected on radial growth rate only.

Analysis of bioluminescence. — Procedures for analysis of bioluminescence have been described previously (Bermudes & al., 1990). Single-spore and polyspore isolates were grown at room temperature on a molasses-yeast extract medium for initial photometric readings, interpreted as luminescent (+) versus non-luminescent (–). Subsequently, the polyspore culture from collection *RHP* 2416 (Illinois) and certain single-spore isolates exhibiting a delay in the onset of luminescence, as well as dikaryon strain, ATCC 66462 used previously (Bermudes & al., 1990), were grown on bread-crumbs agar, and periodically measured photometrically for total luminescence. Colony diameter was recorded at 14 days in order to assure a general comparability of the cultures.

RESULTS

Self-crosses. — For collections *RHP 2416* and *RHP 2536*, ten single-spore isolates were employed in self-crosses, while in collection *RHP 2315*, 13 isolates were used. In collection *RHP 2300* only seven single-spore isolates were established, and all were used in the self-cross. Single-spore isolates were crossed in all combinations. In all cases except *RHP 2300*, a bifactorial mating system was revealed as expected. In collection *RHP 2300*, all self-crosses were incompatible.

Intercollection matings. — When tester strains from the four collections were mated, isolates from collection *RHP 2315* (Japan) were found to be universally compatible with tester strains from all other collections, including *RHP 2300* (where no discernable mating system had been ascertained) (Fig. 1). Aside from intercompatibility, the following observations were made on these matings:

1. In virtually all cases (but see 2315/11 \times 2300/6) relative abundance of clamp-connections in the contact zone was significantly higher than in other areas of the mated colonies.
2. In reciprocal matings between tester strains of *RHP 2315*, *RHP 2416*, and *RHP 2536*, nuclear migration (see above) was always accompanied by hyphal lysis of the recipient strain.
3. Although suggestions of flat and barrage phenomena could be observed, no clear-cut reactions were seen.

Bioluminescence. — As suggested by Macrae (1942) all Japanese isolates were non-luminescent, but a significant number of Illinois single-spore isolates were also non-luminescent (Table I). In some cultures the onset of luminescence occurred slowly and reached relatively low levels of total luminescence (Fig. 2). In the initial survey of luminescence, one isolate (2416-19) showed a diminution in luminescence to below the level of detection of the photometer (approximately 1×10^5 quanta (q) per second) (Table I). In the second more quantified experiment, the same culture exhibited a marked drop in luminescence, but after a shorter growth period, and not to the same low level (Fig. 2). Complete loss and subsequent resumption of luminescence is also sometimes observed in mature (over 4 weeks old) dikaryotic cultures of *P. stypticus* (unpublished observation). Of the single-spore isolates surveyed, all reached average ($n = 3$) diameters of between 40–43 mm in 14 days. Dikaryotic strains ATCC 66464 and 2416-polyspore reached average diameters of 46 and 50 mm respectively.

CONCLUSIONS AND DISCUSSION

The only significant macromorphological differences among the fresh basidiomata was a darker, ruddy color of basidiomata of *RHP 2536*. This could be attributed to cold weather, for the basidiomata were collected in January, just after several days of low temperature. Basidiomata of *P. stypticus* develop slowly, so these were surely exposed to subfreezing temperatures. If this difference is discounted, then all four collections must be placed in the same macromorphological species. Micromorphology of all collections fell within the range described by Miller (1970) for the species.

Macrae's (1942) report implied that specimens from Europe and Asia lacked bioluminescence, and photometric analysis of the Japanese cultures in this study (by DB) has affirmed

Table I. Bioluminescence of Illinois isolates of *Panellus stypticus*.

Isolates from <i>RHP 2416</i>	Day			
	5	9	14	30
polyspore	+	+	+	+
single-spore				
1	-	-	-	-
2	-	-	-	-
3	-	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	-	-	-	-
8	+	-	-	-
9	-	+	+	+
10	-	-	-	-
11	-	-	-	-
12	+	+	+	+
13	+	+	+	+
14	-	-	-	-
15	-	+	+	+
16	+	+	+	+
17	-	+	+	+
18	-	+	+	+
19	-	+	-	+
20	+	+	+	+

this. Single-spore isolates from the Illinois collection, however, varied from non-luminescent to moderately so, with some isolates developing luminescence only after some period of growth (Fig. 2), an unexpected result. Lingle & al. (1990) recently reported on such occurrences, indicating that crosses between such non-luminescent isolates produced luminescent progeny.

Obviously, genes for bioluminescence are independent of those for mating types, for luminescence occurred in the Illinois single-spore isolates regardless of mating types, and the non-luminescent Japanese isolates were compatible with luminescent North American strains. Simply on the basis of supracontinental geographic distribution, loss or suppression of the genes for luminescence must have occurred early, or the origin of the bioluminescent system may have occurred after the separation of North America and Eurasia (c. 200 million years before present), while sexual barriers have yet to be erected.

While in most crosses nuclear migration did not occur beyond the contact zone, in cases where nuclear migration was inferred, it seemed strain-specific rather than governed by mating type genes. Isolate 2315/11, for example, was dikaryotized by most other isolates,

but showed little ability to dikaryotize others. Isolate 2416/7, conversely, dikaryotized almost all its mates while rarely allowing nuclear migration within its own hyphae. Finally, isolate 2300/6 acted as both donor and receptor, and when crossed with 2315/11, produced many more clamp-connections at the periphery of the Petri dish than in the contact zone. In most other crosses, only sporadic nuclear migration was inferred. Although the phenomenon of hyphal lysis in the contact zone occurs in crosses of other similar organisms (i.e. *Xeromphalina*, unpublished data), nuclear migration also seemed linked to hyphal lysis in this study. Mycelium without nuclear migration clamps appeared healthy, with turgid hyphae throughout, while mycelium exhibiting nuclear migration clamps included many lysed hyphae and other hyphae with highly vacuolate contents. Empty hyphae, detected only by residual walls and cross-walls, were common in these areas. The colony margin, however, was usually healthy, with occurrence of clamps at least 250–500 μm from the growing hyphal tips.

		2300				2315				2416				2536			
		4	5	6	8	11	5	3	4	9	7	3	4	7	2	1	11
2300	4					+	+	+	+	+	+	+	+	+	+	+	+
	5					+	+	+	+	+	+	+	+	+	+	+	+
	6					+	+	+	+	+	+	+	+	+	+	+	+
	8					+	+	+	+	+	+	+	+	+	+	+	+
2315	11									+	+	+	+	+	+	+	+
	5									+	+	+	+	+	+	+	+
	3									+	+	+	+	+	+	+	+
	4									+	+	+	+	+	+	+	+
2416	9													+	+	+	+
	7													+	+	+	+
	3													+	+	+	+
	4													+	+	+	+
2536	7																
	2																
	1																
	11																

Fig. 1. Compatibility of *Panellus stypticus* strains. The symbols in each grid represent positive or negative results in mating different *P. stypticus* strains. The central symbol represents compatible mating as discerned by the presence of clamp-connections in the contact zone. The symbols in the upper right and lower left corners indicate whether clamp-connections were found on the opposite side of the inoculum block, thereby indicating nuclear migration.

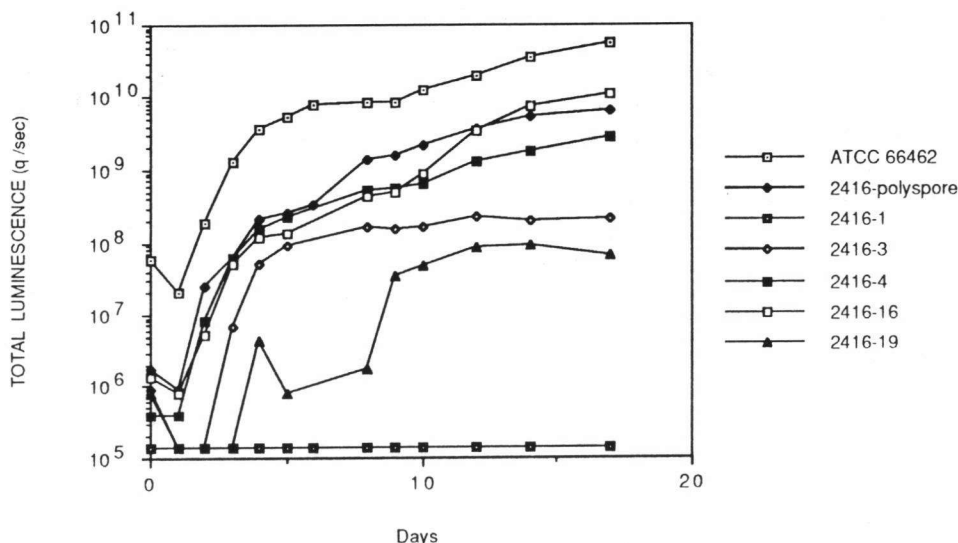


Fig. 2. Total luminescence produced during growth of *Panellus stypticus*. Cultures were grown on bread crumb agar and assayed for total luminescence. Cultures with no detectable luminescence were recorded as less than or equal to the detection limit of the photometer (1.1×10^5 quanta (q) per second).

It is evident that genetically controlled traits (i.e. morphology, sexual compatibility, luminescence) do not necessarily follow the same course of evolution. While variation may be masked in complex 'dikaryon' mycelium (in nature probably comprising more than two nuclear types), such variation is more readily observed in haploid systems (i.e. single-spore isolates). In this study, ability to dikaryotize receptor mycelium is such a strain-specific variable trait, as is the presence or absence of bioluminescence. On the other hand, the cause(s) of apparent absence of luminescence by European, Asian (both monokaryon and dikaryon states) and some North American (monokaryon only) strains of this biological species, cannot be ascertained from this study.

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