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Temperature-Dependent Spore Dimorphism in *Burenella dimorpha* (Microspora: Microsporida)

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ABSTRACT. *Burenella dimorpha*, a microsporidian parasite of the tropical fire ant, *Solenopsis geminata*, produces two morphologically distinct types of spores. The binucleate free spores (spores not bound by a pansporoblast membrane) develop normally at temperatures at least as low as 20°C and as high as 32°C. The uninucleate octospores (spores bound in octets by a pansporoblast membrane), however, develop in a restricted range of temperature. Octospores constituted 35.9% ± 2.6 of the spores in 25 pupae held at 28°C. Raising the temperature to 30°C reduced octospores to <1% of the total spore population. Lowering the temperature to 25° or 22°C reduced the octospore population to 8.5% ± 6.5 or 0.4 ± 0.5, respectively. Inhibition of octospore development was complete at 20°C. In contrast, the octospores of *Vairimorpha necatrix* and *Vairimorpha plodiae* are reported to be abundant at 16°C and 21°C, respectively. The critical event blocked in octospore development may be meiosis, as evidenced by an abundance of binucleate sporonts in the octospore sequence of development, and absence of more advanced sporogonic stages in hosts held at inhibitory temperatures. Free spore size is not affected by temperature although yield may be slightly reduced at elevated temperature.

BURENELLA dimorpha, a parasite of the tropical fire ant, *Solenopsis geminata*, produces two morphologically distinct types of spores. Binucleate free spores (spores not bound by a pansporoblast membrane) develop from disporous sporonts in the hypodermal tissue; uninucleate octospores (spores bound in octets by a pansporoblast membrane) develop from octonucleate sporonts in the fat body. Free spores develop before octospores and predominate in number. Spore dimorphism is a definitive characteristic of the family Burenellidae (5) and also occurs in certain genera of the family Thelohaniidae (4). The tissue specificity of the spore types varies among species.

The effect of temperature on the relative abundance of free spores and octospores was first studied by Maddox (10) in a microsporidiosis of the armyworm, *Pseudaletia unipuncta* (Lepidoptera). This disease was originally diagnosed as a mixed infection involving two new species of microsporidia, *Nosema necatrix* and *Thelohania diazoma* (8). Maddox, however, found that octospore development depended on the temperature at which the host was reared. Octospores constituted 40% or more of the spores in larvae reared at 16°C, but were absent from larvae reared at 32°C or higher. Larvae infected perorally by an apparently pure suspension of free spores (from hosts reared at 32°C) and held at 16°C produced octospores in normal numbers. These observations led Maddox to suggest that "*N. necatrix-T. diazoma*" were possibly a single species.

The dimorphic nature of this microsporidium, which is now named *Vairimorpha necatrix* (12), was experimentally demonstrated by Fowler & Reeves (2) through the use of mechanical as well as thermal and temporal methods to separate the two spore types for transmission studies. Maddox & Sprenkel (11) confirmed temperature-dependent spore dimorphism in *V. necatrix* by serially transmitting infection via pure suspensions of free spores through eight generations of hosts reared at 32°C without loss of the ability to produce octospores in hosts held at lower temperature. In the same paper, Maddox and Sprenkel presented evidence that *Vairimorpha plodiae* ("*Nosema plodiae-Thelohania nana*") and an undescribed microsporidium are also single, temperature-dependent dimorphic species.

Preliminary observations indicated that *B. dimorpha* octospores develop only in a restricted range of temperature. We conducted the study presented here to determine the thermal limits of spore development in this species, and the effects of temperature on spore size and yield. Since the free spores and octospores of *B. dimorpha* develop in different tissues (hypodermal tissues and fat body, respectively), we were also able to investigate whether: a) the fat body is not invaded, b) the fat body is invaded but development is arrested at some point, or c) free spores develop in lieu of octospores in the fat body at temperatures inimical to development of the latter.

MATERIALS AND METHODS

Thermal effects on spore ratios. Laboratory colonies of *S. geminata* were maintained as described by Banks et al. (1). Fourth-instar larvae were removed from a heavily infected laboratory colony and held in miniature nest cells at the desired temperature (20°, 22°, 25°, 28°, 30°, 32° and 35°C) overnight. Humidity was maintained close to 100% by placing wads of wet cotton in the enclosed foraging area of the cell. Larvae that pupated during the first 16 h were discarded as were those that had not pupated during the next 24 h.

A contingent of nurses (young workers primarily care for the immatures) captured from the brood piles was introduced to care for the immatures. These nurses were removed shortly before the end of pupal life (at that time they were no longer needed to groom the pupae) to facilitate the examination and harvest of specimens in advanced stages of infection. The uninfected pupae served as controls to monitor the rate of maturation at the different temperatures. Infected pupae were harvested only after eclosion of the controls when the disease had progressed to near the point of cuticle rupture and sporulation was essentially complete (6). The completeness of sporulation was judged by the percentage of immature spores seen in phase-contrast microscopic examination of suspensions. Spore ratios were determined only for those pupae in which at least 98% of the free spores appeared to be mature.

Spore ratios were determined by homogenizing individual pupae in ca. 1 ml distilled water in a 3-ml glass tissue grinder and examining this extract by phase-contrast microscopy at a magnification of 600×. The concentration of spores in these extracts (0–6 spores/field) was low enough to facilitate counting and study of individual spores. The percentage of octospores was based on the first 200 spores observed.

Spore measurements. Spore measurements were made using an A. E. I. Cook Image-Splitting micrometer calibrated for the microscope used. The spores were immobilized for measurement by trapping them between a layer of 1.5% Noble Agar (Difco Laboratories, Detroit, MI) and the coverslip.

Spore yield. Spore yield was determined by weighing groups of 25 pupae in advanced stages of disease, homogenizing these in distilled water, adjusting the volume appropriately, and counting spores with a hemacytometer using phase-contrast microscopy at a magnification of 300×.

Histology. Tissue specimens from gasters of pupae held at 20° or 32°C until infection was very advanced were embedded in epoxy resin, sectioned, and examined (light microscopy) as described by Jouvenaz et al. (6).

Giemsa stains. Specimens were smeared on acid alcohol-cleaned glass microscope slides, air-dried, and fixed in methanol

TABLE I. Burenella dimorpha *spore yield of pupae of* Solenopsis geminata *reared from larvae at 20°, 28°, or 32°C.*

Temperature (degrees C)	Free spores		Octospores	
	Spores/pupa	Spores/mg	Spores/pupae	Spores/mg
20°	6.7×10^5	3.9×10^5	0	0
28°	6.3×10^5	4.4×10^5	2.7×10^5	2.0×10^5
32°	4.8×10^5	2.7×10^4	0	0

for 5 min. Slides were stained with 10% Giemsa stain in phosphate buffer, pH 7.41 (Fisher Gram Pac Buffer) for 12 min, rinsed in tap water or acidified deionized water (deionized water adjusted to pH 6.8 with acetic acid), blotted with filter paper, and examined microscopically under oil.

RESULTS AND DISCUSSION

Burenella dimorpha free spores appear to develop at all temperatures compatible with host survival. Similar numbers of spores were produced in pupae held at 20° and 28°C; slightly fewer were produced in pupae held at 32°C (Table I). In hosts held at 35°C, ca. 2% of the spores were morphologically aberrant (pairs of spores fused laterally or in tandem, Y-shaped or triangular spores, giant spores, etc.). Brood survival was severely depressed at 35°C, which appears to be very near the upper thermal limit of survival for host and parasite.

The measurements of free spores from pupae held at 20°, 28°, or 32°C were respectively, $3.0 \pm 0.2 \times 6.8 \pm 0.3$ μm, $3.0 \pm 0.1 \times 6.8 \pm 0.3$ μm, and $3.0 \pm 0.1 \times 7.0 \pm 0.4$ μm. Obviously, there were no differences in sizes of free spores.

In contrast to free spores, *B. dimorpha* octospores develop in a rather narrow range of temperature (Table II). Octospore numbers were maximal and most consistent in pupae held at 28°C and were progressively reduced in pupae held at higher or lower temperatures. Raising the temperature only 2° (to 30°C) reduced octospores from 35.9% to ca. 1% of the total spore population, and at 32°C, octospore inhibition was essentially complete. The inhibitory effect of reduced temperatures was almost as pronounced. At 25°C, octospores constituted only 8.5% of the total spore population and at 22°C, ca. 0.4%. Inhibition was complete at 20°C. The optimal temperature for *B. dimorpha* octospore development is thus 28°C, the lower thermal limit is between 20° and 22°C, and the upper thermal limit is ca. 32°C.

The octospores of *V. necatrix* and *V. plodiae* reportedly develop at lower temperatures than do those of *B. dimorpha.* Octospores constituted 40% or more of the *V. necatrix* spores in hosts held at 16°C (10) and the upper thermal limit of octospore development in this species is below 25°C (12). The octospores of *V. plodiae* do not develop at 16°C but are abundant (even dominant) in hosts held at 21°C, and the greatest concentrations occur in diapausing pupae. The upper thermal limit of octospore development in this species is between 27° and 32°C (11).

The restricted range of temperature in which octospores develop is not a liability for *B. dimorpha,* for the behavior of its host insures a rather constant environment. *Solenopsis geminata* is a subtropical, eusocial insect that actively cares for its young (adults are not infected), moving them within a subterranean environment to regulate temperature and moisture. The tumulus of the nest is a solar heating device. In field colonies, octospores typically constitute ca. 25–40% (occasionally fewer) of the spores (5). Thus, the ants are able to maintain their brood very close to 28°C. The various hosts of the *Vairimorpha* spp. are, of course, exposed to ambient temperatures.

TABLE II. *Relative abundance of* Burenella dimorpha *octospores in infected pupae of* Solenopsis geminata[a] *held at various temperatures.*

Temperature (degrees C)	Incubation time (days)	Number of pupae	No. (%) positive for octospores	Percent octospores in all specimens
20°	57	25	0	—
22°	39	15	5 (33)	0.4 ± 0.51
25°	23	25	23 (92)	8.5 ± 6.5
28°	18	25	25 (100)	35.9 ± 2.6
30°	18	25	6 (24)	0.8 ± 1.9
32°	16	25	3 (12)	<0.1
35°	15	10	0	—

[a] 100% positive for free spores at all temperatures.

Neither octospores nor free spores were seen in tissue sections of fat body from pupae held at 20° and 32°C. Octospores were seen occasionally in sections of fat body of pupae held at 22°C. Numerous binucleate sporonts in the octospore sequence of development were seen in Giemsa-stained smears of pupae held at 20° and 32°C; however, no tetra- or octonucleate sporonts were found.

We may only speculate as to the physiological function(s) that are inhibited by extremes of temperature. The critical event in spore type differentiation, however, may well be meiosis. If the nuclei of the binucleate presporogonic vegetative stages of *B. dimorpha* are each diploid, meiosis would produce eight uninucleate, haploid octospores. In the absence of meiosis, a diplokaryotic free spore would develop. Evidence that meiosis does indeed occur in microsporidia has been published by Loubes et al. (9) and Hazard et al. (3). The abundance of binucleate sporonts in the octospore sequence of development (and the absence of more advanced sporogonic stages of this sequence) in pupae held at inhibitory temperatures suggests that octospore sporulation in *B. dimorpha* proceeds only to a point immediately preceding nuclear doubling and quadrupling (meiosis?).

We have used the term "inhibition" to denote the reduction or absence of octospores in fire ant pupae infected with *B. dimorpha* and held at inimical temperatures. Whether octospore development is truly inhibited in the sense of absolute cessation of development or is simply greatly slowed was impossible to determine. It is possible that octospores would develop if it were possible to hold the pupae indefinitely; however, infected pupae rupture in the stage of disease at which our examinations were made (6, 7).

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