

Laboratory investigations of trichomycete prevalence, abundance and fecundity in a *Smittium*-simuliid model

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Abstract: *Smittium*, the most speciose genus of the “gut fungi” (Zygomycota: Trichomycetes), is found attached to the hindgut cuticle of larval aquatic Diptera. *Smittium* spp. colonize several host families (e.g., *Smittium culisetae* in Chironomidae, Culicidae and Simuliidae), but some species appear to be specific to a single host family (e.g., *Smittium morbosum* Sweeney in Culicidae). The specificity of *Smittium* spp. within a host family has been difficult to resolve. This research presents evidence that certain *Smittium* spp. differentially colonize particular species of black fly (Diptera: Simuliidae) hosts as measured by differences in prevalence, abundance and fecundity. Reasons for this differential occurrence and fecundity in hosts are unclear but might include fungal responses to variations in host morphology, physiology, distribution or behavior. Variable fitness of *Smittium* spp., within a suite of available hosts, could be a factor in the diversity of this fungal group.

Key words: axenic cultures, colonization, Harpellales, Simuliidae, symbiosis

INTRODUCTION

Gut fungi (Zygomycota: Trichomycetes) are obligate symbionts of the digestive tracts of various arthropods, particularly aquatic insects (Lichtwardt 1986, Misra 1998, Moss 1998). The largest order, Harpellales, comprise 34 genera, the most species-rich genus being *Smittium* (Legeriomycetaceae) (Lichtwardt et al 2003). Fifty-five species of *Smittium* have been found attached to the hindgut cuticles of several fam-

ilies of aquatic dipteran larvae, as well as larval mayflies (Ephemeroptera) and stoneflies (Plecoptera) (Lichtwardt and Williams 1990, Williams and Lichtwardt 1990, Lichtwardt et al 2001, Alencar et al 2003). Host specificity of *Smittium* usually has been discernible at the family taxon, although some species colonize several host families within an order. For example, *Smittium culicis* Manier has been found in variety of families of the lower flies (Diptera: Nematocera) including Chironomidae, Culicidae, Psychodidae, Simuliidae, Thaumaleidae and Tipulidae (Lichtwardt et al 1999). Advances in axenic culturing techniques (Lichtwardt 1964) have made *Smittium* spp. available for molecular (Sanger et al 1972, Gottlieb and Lichtwardt 2001, White 2002), nutritional (Horn and Lichtwardt 1981), physiological (Williams and Lichtwardt 1972a; El-Buni and Lichtwardt 1976a, 1976b; Horn 1989) and ultrastructural studies (Sato et al 1989).

Black fly (Diptera: Simuliidae) larvae are often one of the most dominant insects in the stream benthos (Cummins 1987, Adler et al 2004). Most larval black flies are filter feeders, using a pair of modified labral fans to remove particles from the water column (Currie and Craig 1987, Crosskey 1990). Larvae passively capture a wide array of food particles of varying sizes, ranging from 0.091 to 350 μm diam (Crosskey 1990), which regularly consists of animal matter, bacteria, detritus, diatoms, filamentous algae and fungal spores (Kurtak 1978, 1979). Larval simuliids worldwide are known to harbor 12 species of *Smittium* (Nelder, McCreadie and Beard unpubl data). Five of these species have been reported exclusively from larval simuliids, while the other seven species are known to colonize additional families of aquatic Diptera.

A *Smittium*-simuliid model was adopted in these experiments because both symbiont moieties can be maintained and manipulated under laboratory conditions. In addition, black flies were selected as the model host because they are a ubiquitous part of the macro-invertebrate stream fauna (Adler and McCreadie 1997).

In this paper we examine the occurrence and fecundity of five species of trichomycetes in various hosts. Occurrence, as used here, has two components. The first is prevalence (i.e., the number of host larvae with gut fungi divided by the number of

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TABLE I. Summary of *Smittium* spp. used in laboratory studies

Trichomycete ^a	Larval host	Host collection site ^b
<i>Sm. culisetae</i> (6810)	<i>S. tribulatum</i> (Simuliidae)	Willard's Pond Outflow, Pickens Co., SC, USA, 34°39.4'N 82°49.2'W
<i>Sm. megazygosporum</i> (6275)	<i>S. tribulatum</i> (Simuliidae)	Willard's Pond Outflow, Pickens Co., SC, USA, 34°39.4'N 82°49.2'W
<i>Sm. morbosum</i> (WS701)	<i>Ochlerotatus triseriatus</i> (Say) (Culicidae)	Woodall Shoals near Chatooga River, Oconee Co., SC, USA: 34°47.8'N 83°18.8'W
<i>Sm. near typhellum</i> (WPA030397)	Unidentified bloodworm (Chironomidae)	"Willard's Pond Annex", Pickens Co., SC, USA: 34°39.3'N 82°49.8'W
<i>Smittium brasiliense</i> (07-052202-03)	<i>Simulium decorum</i> Walker (Simuliidae)	Threemile Creek, Mobile Co., AL, USA, 30°37.4'N 88°10.6'W

^a Numbers in parentheses are designation numbers. *Smittium culisetae* and *Smittium morbosum* are deposited in the United States Agricultural Research Service entomopathogenic culture collection, Ithaca, New York. *Smittium morbosum* and *Smittium near typhellum* are maintained at Clemson University and *Smittium* sp. 1 at the University of South Alabama.

^b All sites were streams, except Woodall Shoals, which was a tree-hole.

hosts examined); it is expressed quantitatively as a percent. The second is relative abundance (i.e., the amount of hyphal growth in gut of the host [McCreadie and Beard 2004]). Accordingly we examined whether occurrence of several species of *Smittium* varies within a single species of black fly and whether occurrence of a single species of *Smittium* varies among several host species of black flies.

In addition to occurrence we also examine trichomycete fecundity, defined here as the number of trichospores produced by thalli attached to the hindgut of the host. Trichospores are the asexual reproductive propagules, and the number of mature trichospores produced by a fungus can be used as a measure of fitness. Here fitness is used in the broad sense (i.e., the probability of contributing to the next generation). The four hypotheses to be tested in this study are: (i) occurrence of different *Smittium* spp. does not vary in a single host species; (ii) fecundity of different *Smittium* spp. does not vary in a single host species; (iii) Occurrence of *Smittium* spp. does not vary among species of hosts; (iv) fecundity of different *Smittium* spp. does not vary among species of hosts. This is the first study to report differential occurrence and fecundity of *Smittium* species among black fly hosts.

MATERIALS AND METHODS

General rearing protocols.—Detailed protocols for host and symbiont maintenance have been outlined by McCreadie and Beard (2003). Hence, only the most pertinent details will be given here. Stock cultures of *Smittium brasiliense* Alencar, Lichtwardt, Ríos-Velásquez & Hamada, *Smittium culisetae* Lichtwardt, *Smittium megazygosporum* Manier & Coste, *Smittium morbosum* Sweeney and *Smittium near typhellum* (TABLE I) were maintained on plates of 3.7 g/L Brain Heart Infusion agar (Difco®) at 23–25 C, with month-

ly transfers to fresh plates; a sterile water overlay was not used on our stock cultures. Hyphal subcultures were transferred (see Lichtwardt 1986 for methods) to new plates 10 d before the start of an experiment. Plates were covered with a sterile water overlay to induce trichospore production, and trichospores were harvested by filtering the overlay through a glass-wool/aquarium-floss plug and centrifuged at 900 g for 10 min (Horn 1989). Trichospore concentration in the resulting suspension was determined with a counting slide (Haemocytometer, improved Neubauer scale). Inocula were aliquots of 4000 trichospores/mL of host-rearing water.

Hosts used for experiments were *Simulium innoxium* Comstock and Comstock (= *Simulium pictipes* Hagen), *Simulium tribulatum* Luggler (= *Simulium vittatum* Zetterstedt cytospecies "IIL-1"), *Simulium verecundum* Stone & Jamnback *sensu stricto* and *Simulium vittatum* Zetterstedt (= *Simulium vittatum* Zetterstedt cytospecies "IS-7") (Adler et al 2004). Eggs of *Simulium vittatum* were obtained from a colony at the University of Georgia (Athens, Georgia). Remaining hosts species were collected as eggs in the field at these locations: *Simulium innoxium*, Six Mile Creek, Pickens County, South Carolina, USA: 34°48.1'N, 82°50.7'W; *Simulium verecundum s.s.* and *Simulium tribulatum*, Threemile Creek, Mobile County, Alabama: 30°37.4'N, 88°10.6'W and 30°37.4'N, 88°0.6'W, respectively. Field-collected eggs were refrigerated (4 C) until needed. Hatching occurred when eggs were placed in aerated water at 22 C. Larval age hereafter refers to the interval after eggs were placed in 22 C water. Larvae were fed fish-food slurry of 4 g of Tetra® fish food suspended in 1 L of water and 1 min of agitation in a blender. All experiments were conducted in Percival® incubators at 22 C with a light/dark regime of 16/8 h. Each treatment container consisted of a 12 cm diam × 11 cm tall round polypropylene plastic container fitted with a lid. Air supplied to container water by air stones created currents simulating the lotic conditions required by larval black flies.

Assessment of relative abundance, fecundity and prevalence.—The abundance of hyphae in a larval host was assessed following a modification of McCreadie and Beard (2003).

TABLE II. Experiments 1–3, Analysis of variance of the mean relative abundance of thalli of four species of *Smittium* in the larval black fly host *Simulium vittatum* reared under laboratory conditions

Trichomycete species	Mean relative abundance (%) of thalli (\pm SE) ^a		
	Experiment 1	Experiment 2	Experiment 3
<i>Smittium brasiliense</i>	46.5 \pm 8.24A ^b	51.8 \pm 7.89A	52.6 \pm 8.44A
<i>Sm. megazygosporum</i>	18.4 \pm 4.78B	12.8 \pm 4.62B	6.7 \pm 2.03B
<i>Sm. morbosum</i>	9.3 \pm 2.16B	10.8 \pm 3.64B	21.1 \pm 5.72B
<i>Sm. nr. typhellum</i>	5.2 \pm 1.80B	5.4 \pm 1.67B	11.6 \pm 4.65B
F value	F _{4,116} = 14.0 ^c	F _{4,116} = 15.7 ^c	F _{4,116} = 12.9 ^c

^a Means of raw percent data given for comparison purposes only. Analysis performed on arcsin-transformed data.

^b For a given experiment, means followed by different letters are significantly different at the individual error rate of $P < 0.00833$.

^c Significant at $P < 0.0001$.

From each experimental container 10 larvae were examined for trichomycetes. Larvae were placed in a drop of tap water under a dissecting microscope. The hindgut was removed, cleared of food, and the area with the densest amount of hypha(e) was viewed at 400 \times through a 10 \times 10 mm ocular grid under phase-contrast microscopy. The number of grid squares that contained one or more hyphal brachlets were counted; relative abundance was expressed as the percentage of grid squares containing hypha(e) to the total number of grid squares covering the hindgut. When fecundity (i.e., mean trichospore production) was the dependent variable of interest (TABLE II), the numbers of grid squares that contained one or more trichospore(s) were counted and the results again expressed as a percent. The term prevalence (percent colonized) refers to the number of *Smittium*-colonized hosts divided by the number of hosts examined.

Experimental protocol.—At the start of each experiment, 3 wk old larvae were transferred to new 1 L plastic containers with 500 mL of aged tap water. Each container held 40 larvae, and 3 mL of fish-food slurry were added to each container daily during each experiment. Larvae were allowed to acclimate in the containers 2 d before being inoculated. Aliquots (inocula) of 4000 trichospores/mL of host-rearing water were used for all experiments. Three control containers of 40 larvae each were maintained. No larvae examined from control containers were colonized by *Smittium* spp. Each treatment was replicated 3–4 times depending on the specific experiment. Four d after inoculation, larvae were removed from containers and held on moist filter paper in Petri dishes at 4 C for 2 d to let the gut void food (McCreadie and Beard 2003). Ten larvae from each container were selected randomly and, depending on the experiment trichomycete relative abundance, prevalence or fecundity was assessed.

Experiments 1–3: Smittium spp. occurrence within a single host. *Simulium vittatum* larvae were exposed to colonization by trichospores of *Smittium brasiliense*, *Smittium megazygosporum*, *Smittium morbosum* and *Smittium nr. typhellum*. Twelve containers were inoculated for each experiment (i.e., 3 containers per treatment level \times 4 [levels] species). Response variables in these experiments were hy-

phal abundance and prevalence. This experiment was repeated three times.

Experiments 4, 5: Fecundity of fungal species in a single host. *Simulium vittatum* larvae were exposed to colonization by *Smittium brasiliense*, *Smittium megazygosporum*, *Smittium morbosum* and *Smittium nr. typhellum*. Twelve containers were inoculated for each experiment (i.e., 3 containers per treatment level \times 4 [levels] species). The response variable in these experiments was fungal fecundity. This experiment was repeated three times.

Experiment 6–11: Occurrence of Smittium culisetae among hosts. For these experiments the prevalence and relative thallial abundance of *Smittium culisetae* were compared among hosts. Experiments were designed as these paired-host comparisons: *Simulium innoxium* versus *Simulium vittatum* (experiments 6, 7, 8), *Simulium verecundum* s.s. versus *Simulium vittatum* Experiment 9) and *Simulium tribulatum* versus *Simulium vittatum* (experiments 10, 11). The number of experiments for each host was determined by the availability of host eggs. For experiments 6–9 each host pair had four treatment levels; four containers with 40 larvae each of species x, four containers with 40 larvae each of species y and four containers with 20 larvae of species x, plus 20 larvae of species y. Mixed containers produced two treatment levels (i.e., species x in the presence of species y, and the inverse, species y in the presence of species x). The response variables were relative thallial abundance and prevalence. Mixed containers were used to determine whether thallial abundance and prevalence in a host was independent of the presence of the other host species. In experiments 10 and 11 *Simulium tribulatum* and *Simulium vittatum* are isomorphic and cannot be separated morphologically; thus, mixed-species containers were dropped from these experiments.

Statistical analyses.—All measures of fecundity and relative fungal abundance (raw percentage data) were transformed into arcsine (arcsin) percents to achieve normal distributions (Quinn and Keough 2002). Mean arcsin percents, for each trial in each experiment, were analyzed with a one-way analysis of variance (ANOVA). Significant differences among treatment means were determined using the Tukey's method of multiple comparisons. For each treatment,

TABLE III. Experiments 1–3, Chi-square analyses of the prevalence of four species of *Smittium* in the larval black fly host *Simulium vittatum* reared under laboratory conditions

Trichomycete species	Mean percent prevalence (95% CI) ^a		
	Experiment 1	Experiment 2	Experiment 3
<i>Smittium brasiliense</i>	80.0 (61.4, 92.3)A ^b	73.3 (54.1, 87.7)A	76.7 (57.7, 90.1)A
<i>Sm. megazygosporum</i>	60.0 (40.6, 77.3)AB	46.7 (28.3, 65.8)AB	30.0 (14.7, 49.4)B
<i>Sm. morbosum</i>	60.0 (40.6, 77.3)AB	43.3 (25.5, 62.6)AB	53.3 (34.3, 71.7)AB
<i>Sm. nr. typhellum</i>	34.5 (17.9, 54.3)B	33.3 (17.3, 52.8)B	40.0 (22.7, 59.4)B
χ^2 ^c	12.5 ^d	9.64 ^d	7.1 ^d

^a 95 percent Confidence Intervals.

^b For an experiment, prevalences followed by different letters are significantly different at the individual error rate of $P < 0.00833$.

^c Chi-square values used to test the independence of prevalence among trichomycete species.

^d Significant at $P < 0.0001$.

an experimentwise adjustment of P -values was made to preserve a family error rate of $P = 0.05$ for all comparisons between treatments (Zar 1996). Chi-square analysis was used to determine whether prevalence was independent of host. Differences between levels within a treatment were analyzed using post hoc comparisons by chi-square analyses with an experimentwise adjustment of P -values (i.e., P -value = $0.05/6 = 0.00833$ for six comparisons).

RESULTS

Experiments 1–3.—Results for mean relative abundance were consistent for all three experiments. *Smittium brasiliense* had significantly higher relative thalial abundance in the larval host *Simulium vittatum* than the remaining three species, *Smittium megazygosporum*, *Smittium nr. typhellum* and *Smittium morbosum* (TABLE II). No significant differences in mean abundance were detected among these latter three *Smittium* species. With regard to prevalence, the only definitive statements that can be made are that *Smit-*

tium brasiliense exhibited a significantly higher prevalence than *Smittium nr. typhellum* in experiments 1, 2 and 3 and that the prevalence of *Smittium brasiliense* was significantly higher than *Smittium megazygosporum* in Experiment 3. As can be seen in TABLE III, there was considerable overlap in prevalence among the species of *Smittium* in the larval host *Simulium vittatum*. Hence, relative abundance and prevalence do not appear to be completely coupled measurements of occurrence.

Experiments 4–5.—In both these experiments it was shown that fecundity, as measured by trichospore production, varied among the four species of *Smittium* examined. The fecundity of *Smittium brasiliense* was significantly higher in the *Simulium vittatum* host than *Smittium nr. typhellum* and *Smittium megazygosporum* in Experiment 4, and fecundity was not significantly different when comparing *Smittium brasiliense* with *Smittium morbosum* (TABLE IV). In Experiment 6, fecundity of *Smittium brasiliense* was significantly higher than both *Smittium morbosum* and *Smittium megazygosporum*, but not *Smittium nr. typhellum*.

TABLE IV. Experiments 4 and 5. Analysis of variance of mean fecundity of four species of *Smittium*, as measured by trichospore production, in the larval host *Simulium vittatum*, under laboratory conditions

Trichomycete species	Mean trichospore production (%)	
	Experiment 4	Experiment 5
<i>Smittium brasiliense</i>	10.0 ± 3.33A ^a	22.3 ± 4.30A
<i>Sm. morbosum</i>	5.8 ± 2.36AB	8.5 ± 4.05B
<i>Sm. nr. typhellum</i>	0.5 ± 0.33B	10.4 ± 3.63AB
<i>Sm. megazygosporum</i>	0.7 ± 0.15B	2.4 ± 0.95B
F _{4,116}	5.89 ^b	6.18 ^b

^a Means with different letters are significantly different at the individual error rate of $P < 0.00833$.

^b Significant at $P < 0.001$.

Experiments 6–11.—Results for these experiments are provided (TABLE V abundance, TABLE VI prevalence). With regard to relative abundance consistent results were noted in experiments 6–9 with no significant differences found between the abundance of *Smittium culisetae* in a host species treated in isolation or with another host species present. In other words no evidence was found suggesting that the presence of one host species affected the abundance of *Smittium culisetae* in the other host (TABLE V). With regard to the comparisons of *Simulium innoxium* and *Simulium vittatum*, some evidence of differences in the abundance *Smittium culisetae* between these larval hosts was found. In Experiment 7 both *Simulium*

TABLE V. Experiments 6–11. Analysis of variance of the mean relative abundance of *Smittium culisetae* thalli in various species of *Simulium* host larvae, reared under laboratory conditions

Species Pairings	Mean relative abundance (%) of thalli ^a		
	Experiment 6	Experiment 7	Experiment 8
<i>S. innoxium</i>	43.7 ± 8.00A ^b	68.6 ± 6.81A	59.1 ± 6.85A
<i>S. vittatum</i>	24.7 ± 6.80A	12.9 ± 3.77B	38.3 ± .59AB
<i>S. innoxium</i> w/ <i>S. vittatum</i>	32.1 ± 7.19A	56.0 ± 5.68A	49.8 ± 7.15A
<i>S. vittatum</i> w/ <i>S. innoxium</i>	19.1 ± 4.92A	21.6 ± 5.10B	22.9 ± 4.85B
F, df, N	2.67, 4, 116	23.18, 4, 156	5.32, 4, 156
P	0.051	<0.001	<0.01
	Experiment 9		
<i>S. verecundum s.s.</i>	51.3 ± 7.86A		
<i>S. vittatum</i>	21.8 ± 4.68B		
<i>S. verecundum s.s.</i> w/ <i>S. vittatum</i>	52.8 ± 6.41A		
<i>S. vittatum</i> w/ <i>S. verecundum s.s.</i>	24.3 ± 4.60B		
F, df, N	8.34, 4, 156		
P	<0.001		
	Experiment 10	Experiment 12	
<i>S. tribulatum</i>	84.6 ± 4.71A	83.9 ± 5.24A	
<i>S. vittatum</i>	22.7 ± 5.95B	37.5 ± 7.97B	
F, df, N	69.1, 2, 78	24.2, 2, 68	
P	<0.001	<0.001	

^a Means of raw data given for comparison purposes only.

^b Prevalences with different letters are significant at the individual error rate of $P < 0.00833$.

TABLE VI. Experiments 6–11, Chi-square analyses of mean prevalence of *Smittium culisetae* thalli in paired species of *Simulium* host larvae, reared under laboratory conditions

Species pairings	Mean percent prevalence (95% CI) ^a		
	Experiment 6	Experiment 7	Experiment 8
<i>S. innoxium</i>	76.7 (57.7, 90.1)A ^b	85.0 (70.2, 94.3)A	85.0 (70.2, 94.3)A
<i>S. vittatum</i>	60.0 (40.6, 77.3)A	50.0 (33.8, 66.2)C	70.0 (53.7, 83.4)A
<i>S. innoxium</i> w/ <i>S. vittatum</i>	66.7 (47.2, 82.7)A	78.9 (62.7, 90.4)B	72.5 (56.1, 85.4)A
<i>S. vittatum</i> w/ <i>S. innoxium</i>	50.0 (31.3, 68.7)A	55.0 (38.5, 70.7)BC	67.5 (50.9, 81.4)A
χ^2	4.59	7.09	3.38
P	0.032	0.008	0.066
	Experiment 9		
<i>S. verecundum</i>	83.3 (65.3, 94.4)A		
<i>S. vittatum</i>	60.0 (43.3, 75.1)A		
<i>S. verecundum</i> w/ <i>S. vittatum</i>	2.4 (8.51, 20.4)B		
<i>S. vittatum</i> w/ <i>S. verecundum</i>	25.0 (12.7, 41.2)B		
χ^2	20.7		
P	<0.001		
	Experiment 10	Experiment 11	
<i>S. tribulatum</i>	97.5 (86.8, 99.9)A	87.5 (73.2, 95.8)A	
<i>S. vittatum</i>	40.0 (24.9, 56.7)B	63.3 (43.9, 80.1)B	
χ^2	19.9	5.68	
P	<0.0001	0.017	

^a 95 percent Confidence Intervals.

^b For an experiment, prevalences with different letters are significant at the individual error rate of $P < 0.00833$ ($P < 0.05$ for trials 10 and 11).

innoxium and *Simulium innoxium* w/*Simulium vittatum* were significantly more abundant than *Simulium vittatum* or *Simulium vittatum* w/*Simulium innoxium*. In Experiment 8 both *S. innoxium* and *Simulium innoxium* w/*Simulium vittatum* were significantly more abundant than *Simulium vittatum* w/*Simulium innoxium*. Little evidence was found suggesting that the prevalence of *Smittium culisetae* varied among the two hosts *Simulium innoxium* and *Simulium vittatum*. In only one case (Experiment 7) was any significant difference found and this was only between *Simulium innoxium* and *Simulium vittatum*.

In Experiment 9, *Simulium verecundum* s.s. had significantly higher thallial abundance of *Smittium culisetae* than *Simulium vittatum*. Chi-square analysis revealed significant prevalence differences between and within host-species pairs.

In experiments 10 and 11, *Simulium tribulatum* had significantly higher thallial abundance of *Smittium culisetae* when compared to *Simulium vittatum* in both trials. Prevalence of *Smittium culisetae* also was significantly higher (chi-square analysis) in *Simulium tribulatum* than in *Simulium vittatum*.

DISCUSSION

As shown by measures of spore production, prevalence and relative thallial abundance, *Smittium* spp. exhibit differential occurrence and fecundity among different species of larval black flies. It could be argued that *Simulium vittatum* was a more suitable host for *Smittium brasiliense* as shown by the other three species of *Smittium* exhibiting lower fecundity, prevalence and relative thallial abundance exhibited within this host. Except for *Smittium brasiliense* other fungal species used here are naturally and more commonly found in nonsimuliid hosts (i.e., *Smittium megazygosporum* in Chironomidae, *Smittium morbosum* in Culicidae, *Smittium* nr. *typhellum* in Chironomidae [Lichtwardt et al 2004]). The isolation of *Smittium megazygosporum* from *Simulium tribulatum* (TABLE I) and its low level of sporulation in our simuliid-host experiments might indicate that this species of *Smittium* was originally from a substandard host (Beard and Adler 2000). As reported by Williams and Lichtwardt (1972b), trichomycete isolates more readily colonize those hosts that are taxonomically similar to the original isolate's host.

In experiments 6–8, no significant difference was found in *Smittium culisetae* relative thallial abundance and prevalence between *Simulium vittatum* and *Simulium innoxium*. The physiology of the black fly gut and the morphology of the labral fans were not studied here, and the following conjecture is an attempt to explain the observed results. The number

of primary fan rays or the surface area of the labral fans might be related to the ability of a black fly larva to trap certain particle sizes (Davies 1966, Chance 1970). For example, a lack of difference between these two host species, in terms of relative abundance and prevalence, might be related to their similar labral-fan morphologies. Morphology of labral fans often is related to the speed of the stream in which a particular black fly species is typically found (Malmqvist et al 1999) and by the types of food ingested by certain species in specific streams (Carlsson 1962). These two species occupy similar habitats in nature (i.e., fast flowing or headwater streams); therefore they might have similar labral-fan morphologies (Nelder unpubl data).

The significantly higher prevalence and relative thallial abundance of *Smittium culisetae* in *Simulium tribulatum*, compared to *Simulium vittatum*, is interesting because these two host-species are morphologically indistinguishable as larvae (cryptic species). However over most of their geographical ranges they are ecologically distinct (Adler and Kim 1986). In nature *Simulium vittatum* inhabits streams originating from springs or streams that are generally pristine and unpolluted, whereas *Simulium tribulatum* is found in a wider range of stream types (Adler and Kim 1984, 1986). Therefore these results could indicate an ecological or behavioral (preference for certain streams) relationship for differential abundance of *Smittium* spp. in the hosts.

Smittium spp. might be better adapted to *Simulium tribulatum*, *Simulium verecundum* s.s. and their corresponding natural habitats, compared to *Simulium innoxium* and *Simulium vittatum*. *Simulium verecundum* s.s. and *Simulium tribulatum* occupy the Mountain, Piedmont, Sandhills, and Coastal Plain ecoregions of the southeastern USA, while *Simulium vittatum* and *Simulium innoxium* occupy the Mountain ecoregion of the same geographical area (McCreadie and Adler 1998). In general *Simulium verecundum* s.s. and *Simulium tribulatum* had higher prevalences and relative thallial abundance of *Smittium culisetae* than *Simulium vittatum* and *Simulium innoxium* and might be more suitable hosts for *Smittium* species.

Smittium spp. isolated from, and traditionally found in, nonsimuliid hosts can colonize larval black flies. We propose that the *Smittium* spp. investigated here can colonize across families of larval Diptera and that their host specificity can be recognized only at the ecological, rather than a taxonomic, level of host recognition (i.e., "filter-feeding" insect larvae). Williams and Lichtwardt (1972a) performed host specificity studies using axenic cultures of *Smittium* spp. and a larval-mosquito host (*Aedes aegypti* Linnaeus). Their semiquantitative data were not subject-

ed to statistical analysis; consequently, no definitive conclusions concerning differential occurrence could be made. Differences in occurrence of *Smittium* spp. likely are associated with the behavior, habitat, morphology and physiology of the host larval black fly (Williams and Grigg 1990).

Although speculative, we hypothesize that primordial *Smittium* spp. might have been faced with periods in which favorable hosts were not available for colonization. A *Smittium* species' ability to switch hosts and habitats is an important aspect of their survival and diversity. The ability to colonize a variety of hosts, especially across families, could be a useful trait for long-term survival. With this in mind, colonization of multiple families of hosts might be a primitive trait, whereas a restricted range of hosts is a specialized or derived trait.

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