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Context-dependent symbiosis between black flies (Diptera: Simuliidae) and trichomycete fungi (Harpellales: Legeriomycetaceae)

John W. McCreadie, Charles E. Beard and Peter H. Adler

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The context-dependent nature of a symbiotic relationship between a trichomycete fungus (*Smittium culisetae*) and a larval black fly (*Simulium vittatum*) is demonstrated in the present study. No significant difference was found between the size of larvae colonized by trichomycetes and those free of trichomycetes, regardless of the trichospore dosage or initial age of the larvae. This trend suggests that the trichomycete has no detectable effect on host fitness, indicating a commensalistic relationship. However, in half of the experiments, stressed (i.e. starved) larvae exposed to trichospores at a dosage of 20 000 spores ml^{-1} had significantly higher survival than did trichomycete-free larvae, indicating a mutualistic relationship. Trichomycetes in adult female black flies can replace the ovaries. The symbiotic association between trichomycetes and simuliids, therefore, is dynamic: commensalistic when larvae are well fed, mutualistic when larvae are starved, and parasitic in adults. The trichomycete-black fly relationship represents a rare case of symbiosis shifting among three states.

J. W. McCreadie, Dept of Biological Sciences, Life Sciences Building Rm 124, Univ. of South Alabama, 307 University Blvd., Mobile, AL 36688-0002, USA (jmccread@jaguar1.usouthal.edu). – C. E. Beard and P. H. Adler, Dept of Entomology, Soils, and Plant Sciences, 114 Long Hall, Clemson Univ., Clemson, SC 29634-0315, USA.

Understanding ecosystem functions often has relied on simple descriptions of species interactions; however, the outcome, nature, and intensity of species interactions are strongly influenced by the abiotic and biotic surroundings (Birch 1953, Price et al. 1986, Douglas 1998). Therefore, characterizations of species interactions (e.g. parasitism as negative/positive and competition as negative/negative) might not be possible without reference to the conditions under which these interactions occur. The few studies on the conditional nature of species interactions have focused on competition, predation, and parasitism (Hutchinson 1961, Schall 1992, Hanski and Henttonen 1996). The context-dependent nature of mutualism has been appreciated more recently (Cushman and Whitham 1989, Breton and Addicott 1992, Gaume et al. 1998, Markham and Chanway 1999).

Symbiosis in the classic sense and for the purpose of our study includes parasitism, mutualism, and commensalism (Boucher 1985, Sapp 1994), although some authors restrict the term to mutualism and commensalism (Pianka 1994). We suggest that in many cases, parasitism and commensalism are the endpoints of a continuum of effects that two species experience when living in close association with one another. Furthermore, the specific nature of symbiotic interactions can depend on the environmental conditions under which the organisms are found.

Trichomycetes (Zygomycota) are cosmopolitan filamentous fungi that live in the guts of various arthropods such as larval black flies (Lichtwardt 1986, 1996). Although trichomycetes are common in aquatic insects, their ecology is little known. Hosts are

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colonized by trichomycetes after ingesting trichospores (Lichtwardt 1996). Following trichospore germination, young thalli attach to the peritrophic matrix in the larval midgut (Harpellaceae) or to the hindgut cuticle (Legeriomycetaceae). Once attached and growing, thalli produce new trichospores, often within 22 hours (Williams and Lichtwardt 1972). Trichospores are then shed into the gut lumen and exit the host via the anus. When hosts molt, hindgut thalli are shed with the hindgut lining (Lichtwardt and Williams 1988). Sexual reproduction involving zygospores is known in some species (Lichtwardt 1996).

Although trichomycetes are obligate inhabitants of the arthropod gut, the responses of the host to trichomycete colonization are largely unknown. The association often is described as commensalistic (Lichtwardt 1986, 1996), although experimental demonstrations are scant and occasional cases of mutualistic or parasitic associations have been noted (Misra 2001). Larvae of the mosquito *Aedes aegypti* (L.) under nutritional stress might benefit from the presence of the trichomycete *Smittium culisetae* Lichtwardt (Horn and Lichtwardt 1981), although no statistical analysis was conducted between treatment groups, warranting caution in the interpretation. In contradistinction, some trichomycetes can be lethal to their hosts. *Smittium* (probably *culisetae*), for example, induces mortality rates exceeding 80% in *Anopheles gambiae* (Coluzzi 1966). Similar mortality rates were found for various species of mosquitoes infected with *Smittium morbosum* Sweeny (Dubitskii 1978, Sweeney 1981, Shimada et al. 1995). Trichomycetes also can be detrimental to adult female black flies, replacing eggs with fungal cysts (Undeen and Nolan 1977, Taylor 1992, Lichtwardt 1996).

The purpose of our study was to determine the context-dependent nature of a symbiotic relationship under specific experimental conditions, using a trichomycete-black fly model. In the wild, larval black flies are hosts of a number of symbionts (McCreadie and Adler 1999, Adler et al. 2004). To eliminate the confounding effects of unwanted symbionts, experiments were conducted using a symbiont-free laboratory colony under controlled laboratory conditions. Given that the trichomycete requires the host to develop and reproduce, we assumed the relationship with the host would remain positive. We therefore focused on the effect of the trichomycete on host fitness and short-term survival. We tested the hypotheses that 1) colonization by the trichomycete influences host fitness, as measured by head capsule size, and 2) the nature of the relationship differs with environmental conditions, specifically food availability to the host.

Material and methods

Maintenance of fungal cultures and inoculum preparation

The trichomycete in our experiments was *Smittium culisetae* (USDA-ARS Collection of Entomopathogenic Fungal Cultures in Ithaca, New York, USA, ARSEF 6810). The isolate was obtained from a larva of the black fly *Simulium tribulatum* (formerly *Simulium vittatum* cytospecies IIIL-1; Adler et al. 2004) collected from a temporary pond outflow (USA, South Carolina, Pickens County, Clemson University, 34°39.4′N 82°49.2′W). Stock cultures were maintained on plates of 3.7 g l^{-1} Brain Heart Infusion agar (Difco 0037-15-0) at 23–25°C, with monthly transfers to fresh plates; a sterile water overlay was not used on stock cultures. Hyphal subcultures were transferred (see Lichtwardt 1986 for methods) to new plates 10 days before the start of an experiment. After 6 days, a sterile water overlay was added to enhance trichospore formation. Four days later, trichospores were harvested by filtering the overlay through glass wool to remove mycelia. The resulting trichospore suspension was centrifuged at 900 g for 10 min and the trichospore 'plug' was rinsed once in water (McCreadie and Beard 2003). Trichospore concentration in the resulting suspension was determined using a counting slide (Hemacytometer, improved Neubauer scale). Depending on the particular experiment, spore suspension sufficient to achieve 4000–20 000 trichospores ml^{-1} of host rearing water was added to the treatment containers. The 4000 spores ml^{-1} dosage was based on our preliminary laboratory trials to achieve high, though not atypical, levels of trichomycete infestations, similar to those in the field (Beard 2002).

Host maintenance

Eggs of *Simulium vittatum* (formerly *Simulium vittatum* cytospecies IS-7; Adler et al. 2004) were obtained from a colony at the University of Georgia (USA, Georgia, Athens). This colony is free of fungi (e.g. trichomycetes), nematodes, and microsporidia (Beard and Adler 2000). Although this colony is 20 years old, recent cytogenetic analysis (Brockhouse and Adler 2002) showed that the level of heterozygosity in the colony is comparable to that in wild populations, indicating that genetic variability has not been compromised during colonization. For each experiment, eggs were transferred from moist storage at 4°C to 1 l rearing containers with 500 ml of aged tap water (McCreadie and Beard 2003). Hatching occurred when eggs were placed in 22°C water, which was similar to the water temperature for the parent colony. Larval age, therefore, refers to the time from when eggs were placed in 22°C water. Aeration was supplied by aquarium pumps fitted with

AS2 Sweetwater® airstones. Larvae in each container were fed a daily ration of 10 ml of a fish-food slurry (stock: 4 g of Tetra® fish food suspended by blending in 1 l of distilled water, McCreadie and Colbo 1991).

All experiments were conducted at 22°C in 5-shelf Precival® incubators, with a 16/8 h light/dark regime. Each incubator was supplied with an external Sweetwater® 5.5 CFM pump and was capable of housing 60 treatment containers. Air supplied to container water created currents, simulating the running water required by larval black flies. Each treatment unit consisted of a 12 cm (diameter) $\times$ 11 cm (height) round polypropylene 1 l plastic container fitted with a screw-top lid (McCreadie and Beard 2003).

Experimental protocol

Host suitability experiments (experiments A and B)

There is always a concern whether animals used in the laboratory respond in a manner similar to that of their wild counterparts. To examine this potential concern, the response by wild collected *Simulium innoxium* to infection with *S. culisetae* was compared to the response of our laboratory colony of *Simulium vittatum*. Eggs of *S. innoxium* were collected from Six Mile Creek, South Carolina, on 12 June 2001, and maintained on ice until the start of the experiment. Eggs of both species were reared to 3 week old larvae as described above. Three replicates of 20 larvae for each species (i.e. treatment = host) were used in each experiment. At the start of the experiment, trichospores were applied at a rate of 4000 spores ml^{-1} of rearing water. The rearing water was changed every two days and re-dosed (McCreadie and Beard 2003). This experiment was terminated after six days and then repeated.

For each experiment and treatment (host), 10 larvae were randomly selected from each replicate and the relative abundance of hyphae assessed as follows. Larvae were placed in a drop of water under a dissecting microscope. The hindgut then was removed and cleared of food. With phase-contrast microscopy, the posterior colon was viewed at 400 $\times$ through a 10 mm $\times$ 10 mm ocular grid, and relative abundance of hyphae was estimated following the procedure of McCreadie and Beard (2003). Landmarks were used to provide consistency in location of each region of the hindgut examined (McCreadie and Beard 2003). Hence, the response of each host to infection was measured as relative abundance. Larvae of *S. innoxium* reared from wild eggs were also examined to ensure that nematodes, microsporidia, and fungi (including trichomycetes) were not present. These preliminary background experiments were referred to as experiments A and B to separate them from the main body of experiments to follow.

Fitness (experiments 1–6)

Our intent was to determine if larval black flies respond to the long-term presence of trichomycetes. Size of last-instar larvae was the response variable and was considered an indirect measure of fitness.

At the start of each experiment, 40 trichomycete-free larvae from the stock population were added to each of 12 experimental containers with 500 ml of aged tap water. Six containers served as controls (not dosed) and the remaining six containers served as treatments (dosed). Although larval age varied among experiments, age within each experiment was constant. After a 24 h acclimation period, six containers were dosed with spores. All containers were provided daily with 3 ml of fish-food slurry. In all experiments, treatment water was changed and dosed with trichospores every 2 days until termination of the experiment (Horn 1989 and others have typically dosed larvae once during experiments). Six or eight days after the start of the experiment, three control and three treatment containers were randomly removed. Ten larvae from each container were selected randomly and placed in a drop of tap water under a dissecting microscope. The hindgut was removed and cleared of food. With phase-contrast microscopy, the posterior colon was viewed at 400 $\times$ through a 10 mm $\times$ 10 mm ocular grid, and relative abundance of hyphae was estimated following the procedure of McCreadie and Beard (2003). This procedure verified that controls were not contaminated and that larvae in treatment containers had trichomycetes. For the remaining containers, larvae were removed and stored in 70% ethanol as they reached the final instar. Each experiment was terminated when all larvae reached the final instar, after which 10 larvae from each container (n = 60 total larvae) were removed randomly. Each larval head capsule was placed in a depression slide with glycerin, and the distance between the antennal buttresses (McCreadie and Colbo 1990) was measured under a dissecting microscope fitted with an ocular micrometer.

Experiments 2, 4, and 6 were repeats of experiments 1, 3, and 5, respectively. Larval ages for experiments 1 and 2 were 22 d. The larvae were dosed with trichospores every 2 days at a rate of 4000 spores ml^{-1} of rearing water. Younger larvae (14 d of age) were used in experiments 3 and 4. Because these larvae were smaller, 8 d were allowed before removal of the first six (3 control, 3 treatment) containers; larvae otherwise would have been too small to examine for fungi. Containers in these experiments were dosed at 4000 spores ml^{-1}. Larvae in experiments 5 and 6 were 20 d old, and containers were dosed at 8000 spores ml^{-1}.

Stress and host response to fungi (experiments 7–18)

Here our goal was to determine if the nature of the symbiosis varied with host condition. The response variable was larval survival after a 6-day period; host

conditions were fed and starved. Twelve experiments (7–18) were conducted: 6 treatment containers were dosed at 4000 spores ml^{-1} of water in 6 experiments, and 6 were dosed at 20 000 spores ml^{-1}. For each experiment, mid-instar trichomycete-free larvae were removed from the stock population and 40 larvae were added to each of 16 containers with 500 ml of aged tap water. The experiment involved a 2×2 factorial design with four replicates per cell. The two levels for the first treatment were trichomycete-positive larvae and trichomycete-free larvae. At the start of each experiment, 8 containers were dosed with trichospores (either 4000 or 20 000 spores ml^{-1}, depending on the experiment) and the remaining 8 containers were not dosed. The two levels for the second treatment were starved larvae and fed larvae. Starved larvae were not fed over the 6 d experiment. Fed larvae were provided with a daily 3 ml ration of fish-food slurry. Thus, the four experimental cells or groups were i) fed-infected larvae; ii) fed-uninfected larvae; iii) starved-infected larvae and; iv) starved-uninfected larvae.

After 6 d, survival in each container was recorded. In all experiments, 20 larvae from the control containers and 20 from the dosed containers were removed randomly and screened for trichomycetes to ensure that controls were not contaminated and that larvae in treatment containers were infected. To determine if the relative abundance of hyphae differed between fed and starved larvae, containers from three experiments with 4000 spores ml^{-1} and three with 20 000 spores ml^{-1} were selected randomly; five larvae from each dosed container then were assessed for the relative abundance of hyphae in the posterior colon, using an ocular grid.

Statistical analysis

All statistical tests were considered significant at $P < 0.05$ and followed the methodology of Zar (1996). Relative abundance data (in host suitability experiments) were analyzed as a one-way analysis of variance, with host species the main effect. A separate analysis of variance was conducted for each gut location (posterior colon, rectum). Because data were expressed as a percentage, they were arcsine transformed (Zar 1996). Head-capsule comparisons between larvae with and without trichomycetes were made using a t-test. Survival data were analyzed using a two-way analysis of variance on arcsine-transformed percentages, with both factors considered fixed. Of particular interest was the interaction between food supply and fungal occurrence. Hence, Tukey multiple comparisons were conducted for all combinations of both factors in those analyses with significant terms (Zar 1996). Differences in hyphal abundance between fed and starved larvae were detected using a t-test on acrsine-transformed percentages.

For each dose (4000 and 20 000 spores ml^{-1}), the results of all six experiments were pooled and analyzed as single data sets. To avoid violating the assumptions associated with pooling experiments (Sokal and Rohlf 1981), a randomization approach was adopted (Manly 1991). As each experiment was a 2×2 design, six comparisons among groups were possible (i.e. $k = [n^2 - n]/2$, where k = total number of comparisons among groups, and n = number of groups). Hence, for each dose, all larvae from each group (e.g. starved-infected larvae) at the end of all six experiments were classified as either alive (1) or dead (0). For each paired comparison (e.g. starved-infected versus starved-uninfected), larval data were pooled and randomly reassigned to each of the two groups being compared. The difference in the number of live larvae between the two groups was the test statistic. By repeating this procedure 5000 times, a distribution for the test statistic, under the null hypothesis of no significant difference between the two groups, was generated. The actual observed difference in the number of surviving larvae was compared with the generated distribution; if the p value of the observed survival was low, the observation was judged significant. Because a total of six comparisons was needed for each dose, an experiment-wise adjustment of the P value was made (Zar 1996) from 0.05 to 0.008 (i.e. 0.05/6).

Results

The relative abundance of *S. culisetae* did not differ significantly between *S. vittatum* and *S. innoxium* in both experiments (Table 1); the results also were consistent between gut locations (i.e. posterior colon and rectum). Accordingly, the response of colony-derived *S. vittatum* to infection with *S. culisetae* is similar to that of wild-collected *S. innoxium* and, by extrapolation, is a reasonable representation of wild responses in general.

The head-capsule sizes of larvae with and without trichomycetes did not differ significantly regardless of the trichospore dosage or the initial age of the starting larvae (Table 2). We interpret this result to indicate that *S. culisetae* had no detectable effect on host fitness under the conditions in our experiments.

In all experiments, starved larvae were stressed, as indicated by a significant food-treatment effect in all experiments (Table 3). The effect of starvation is illustrated by comparing, within each experiment, the survival of fed larvae (with and without trichomycetes) with that of starved trichomycete-free larvae.

At a dose of 4000 spores ml^{-1}, five of the six experiments (7, 8, 9, 11, 12) showed that the fungal treatment effect was not significant. Thus, even under conditions of starvation, the presence of established

Table 1. Mean relative abundance of *Smittium culisetae* in the posterior colon and rectum of two species of black fly larvae. *Simulium vittatum* was taken from a colony, whereas *Simulium innoxium* was collected in the wild.

Experiment[1]	Location	Host	P	F	Mean relative abundance
A	Colon	*S. vittatum*	0.158	2.04	76.7
		S. innoxium			66.8
	Rectum	*S. vittatum*	0.297	1.11	66.3
		S. innoxium			58.8
B	Colon	*S. vittatum*	0.768	0.09	66.3
		S. innoxium			64.2
	Rectum	*S. vittatum*	0.201	1.67	54.0
		S. innoxium			44.7

[1]The dosage used in both experiments was 4000 trichospores ml^{-1} of larval rearing water.

hyphae had no adverse effects on survival. However, the remaining experiment (10) at 4000 spores ml^{-1} had a significant food × fungus interaction. A multiple comparison of the four treatment means showed that starved-infected larvae had a survival rate (78.8%) lower than that of fed groups (91.3–93.8%), but a survival rate higher than in the starved-uninfected group (65.0%). In this case, the presence of the established hyphae under conditions of starvation increased survival over starved-uninfected larvae.

At a dose of 20 000 spores ml^{-1}, four of the six experiments had a significant interaction (food × fungus) and three of the four analyses of variance also showed significant fungal treatment effects. The multiple comparisons of these experiments indicated that under conditions of starvation, larvae with established hyphae had significantly higher survival compared with starved-uninfected larvae, in three of the six experiments (Table 3).

A randomization analysis from the experiments at 20 000 spores ml^{-1} was based on pooled survival of starved-infected larvae and starved-uninfected larvae. The probability of different values of the test statistic (difference in the number of surviving larvae between groups), given 940 live larvae in each group at the start of the experiments, is shown in Fig. 1. Similar analyses and probability distributions (not shown) were run for each of the remaining five comparisons among treatments. These analyses showed that survival was highest in fed larvae, regardless of whether they were infected or uninfected. Survival in starved-infected larvae was significantly lower than in fed larvae but significantly higher than in starved-uninfected larvae.

An example of a randomization analysis, based on pooled survival of starved-infected larvae and starved-uninfected larvae in experiments at 4000 spores ml^{-1}, indicated no significant difference in survival (Fig. 1). Similar analyses and probability distributions (not shown) were run for each of the remaining five comparisons among groups. The results (similar to those in Table 3) indicated that the presence of *Sm. culisetae* had no effect on host survival.

Table 4 provides estimates of the relative abundance of hyphae in fed and starved larvae for three experiments at dosages of 4000 and 20 000 spores ml^{-1} of larval rearing water. In all cases, the abundance of fungal

Table 2. Head-capsule sizes of last-instar larvae of the black fly *Simulium vittatum* with and without the trichomycete *Smittium culisetae*. P values were obtained from t-tests.

Experiment	Age of larvae (days)	Dosage[1]	Mean ± SE relative[2] abundance of hyphae in infected larvae	Mean ± SE head-capsule size		p
				Larvae with trichomycetes	Larvae without trichomycetes	
1	22	4000	58.6 ± 6.6	0.53 ± 0.007	0.54 ± 0.002	0.40
2	22	4000	70.7 ± 6.2	0.51 ± 0.005	0.52 ± 0.005	0.21
3	14	4000	87.0 ± 5.5	0.52 ± 0.005	0.52 ± 0.005	0.80
4	14	4000	61.4 ± 6.7	0.53 ± 0.004	0.53 ± 0.008	0.54
5	22	8000	51.1 ± 6.4	0.52 ± 0.007	0.52 ± 0.007	0.51
6	22	8000	73.2 ± 6.4	0.53 ± 0.004	0.53 ± 0.004	0.93

[1]Number of trichospores ml^{-1} of larval rearing water.
[2]Relative abundance of hyphae was measured following the method of McCreadie and Beard (2003). No infected larvae were found in control containers.

Table 3. Short-term survival of the larval black fly *Simulium vittatum*. Given for each experiment is both the analysis of variance (Anova) and the Tukey multiple comparisons for both levels of the fixed factors: fungus (presence, absence) and food (fed, starved).

Experiment	Dosage[1]	Anova			Multiple comparisons of mean % survival[2]			
		Treatments	F	p	fed/ infected	fed/ uninfected	starved/ infected	starved/ uninfected
7	4000	food	89.89	<0.001	87.5 a	92.5 a	30.6 b	20.0 b
		fungus	0.44	0.520				
		food × fungus	2.61	0.132				
8	4000	food	74.33	<0.001	98.5 a	96.3 a	51.9 b	42.5 b
		fungus	0.09	0.764				
		food × fungus	1.20	0.279				
9	4000	food	147.24	<0.001	95.0 a	93.8 a	32.5 b	31.5 b
		fungus	0.01	0.942				
		food × fungus	0.02	0.885				
10	4000	food	77.30	<0.001	91.3 a	93.8 a	78.8 b	65.0 c
		fungus	2.84	0.118				
		food × fungus	10.02	0.008				
11	4000	food	54.76	<0.001	91.3 a	96.3 a	47.5 b	55.0 b
		fungus	1.88	0.195				
		food × fungus	0.11	0.746				
12	4000	food	30.28	<0.001	93.1 a	93.1 a	70.0 b	66.8 b
		fungus	0.13	0.723				
		food × fungus	0.05	0.830				
13	20 000	food	54.01	<0.001	91.3 ab	95.0 a	72.5 b	31.8 c
		fungus	5.19	0.042				
		food × fungus	12.11	0.005				
14	20 000	food	118.6	<0.001	92.5 a	96.3 a	81.9 b	53.1 c
		fungus	12.7	0.004				
		food × fungus	37.0	<0.001				
15	20 000	food	65.83	<0.001	80.0 a	88.6 a	41.4 b	20.0 b
		fungus	0.23	0.639				
		food × fungus	6.97	0.022				
16	20 000	food	195.92	<0.001	92.5 a	82.5 a	31.9 b	3.8 c
		fungus	23.13	<0.001				
		food × fungus	4.78	0.049				
17	20 000	food	247.47	<0.001	81.9 a	85.0 a	22.5 b	12.5 b
		fungus	1.30	0.277				
		food × fungus	4.01	0.068				
18	20 000	food	40.12	<0.001	90.6 a	96.9 a	58.8 b	53.1 b
		fungus	0.49	0.497				
		food × fungus	1.91	0.192				

[1]Number of trichospores ml^{-1} of larval rearing water.
[2]Statistical analysis was performed on arcsine-transformed data; however, for comparative purposes, percentages are presented. For each experiment, means with different letters are significantly different at a family error rate of $P < 0.05$.

hyphae was significantly ($P < 0.001$) higher in starved larvae than in fed larvae.

Discussion

A certain level of concern always exists as to the degree to which laboratory-based experiments reflect conditions, processes, and outcomes in the field. The colony larvae that we used had levels of genetic diversity comparable those of conspecific field populations (Brockhouse and Adler 2002). Additionally, a wild-collected species (*S. innoxium*) responded to colonization by trichomycetes in a manner identical to that of our colony population. We, therefore, are confident our results indicate that the specific nature of symbiotic interactions can depend on the environmental conditions under which the organisms are found.

Given the lack of significant differences in size between larvae with trichomycetes versus those without trichomycetes, we suggest that, under the conditions in our experiments, the relationship between fungus and host can be described tentatively as commensalistic — tentatively because more subtle fitness characters of the host, such as adult survival and gamete viability, could be influenced by trichomycetes during larval development. Although head-capsule size is an indirect measure of fitness, the strong positive relationship between the size of adult female black flies and fecundity is well established (Colbo and Porter 1981). Hence, large larval females would produce large adult females with greater fecundity. Larger male black flies are more successful in mating with refractory females (Edman and Simmons 1988). Generally, in insects, increased male size trans-

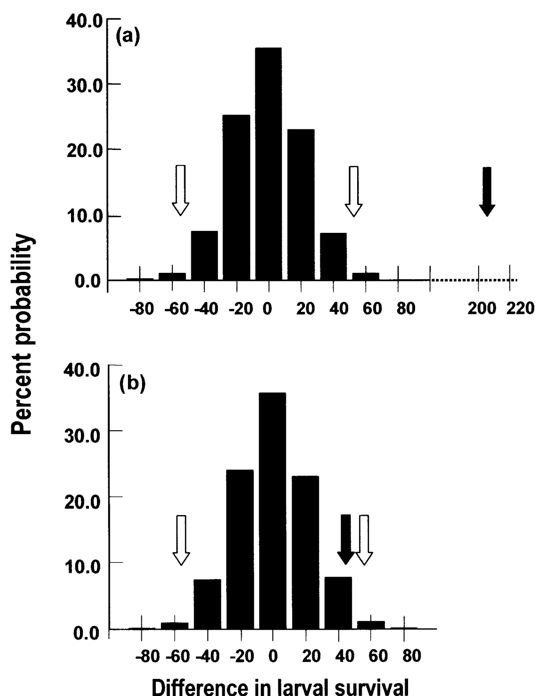

Fig. 1. An example of the results of 5000 Monte Carlo simulations for the pooled survival experiments conducted at 20 000 spores ml^{-1} (Fig. 1a) and 4000 spores ml^{-1} (Fig. 1b). Each graph shows an example of the probability distribution of the test statistic (i.e. the difference in the number of surviving larvae between a pair of treatment groups). In the examples shown, the two treatment groups compared were i) starved-infected larvae and ii) starved-uninfected larvae. For each dosage, a total of six Monte Carlo analyses (remaining five not shown) were conducted (i.e. four treatment groups produce six pairwise comparisons). Open arrows indicate critical values of the test distribution; closed arrows indicate values of the observed test statistic (i.e. the actual difference in larval survival between two treatments).

lates into greater mating success (Thornhill and Alcock 1983).

Larval survival was not compromised by the presence of trichomycetes in any of the 12 survival experiments. In all treatments in which larvae were fed, no significant difference in survival between larvae with and without trichomycetes was detected, regardless of spore dosage. However, under stressed conditions (i.e. starved), larvae exposed to 20 000 spores ml^{-1} had significantly higher survival than did trichomycete-free larvae in three of the six experiments and for the pooled data at this dosage. Given these results, we argue that the relationship between *Sm. culisetae* and *S. vittatum* is mutualistic under conditions of host stress. The basis of this mutualistic relationship is unknown, though we suspect it is nutritional (Horn and Lichtwardt 1981). For example, in all cases, the abundance of hyphae was significantly higher in starved larvae than in fed larvae. More hyphae in stressed larvae might have produced sufficient vital nutrients to increase survival. Several field studies have provided strong evidence that the quality or quantity of food varies within a drainage basin and that larvae exposed to an inferior food supply are smaller than those exposed to a superior food supply (Colbo 1982, McCreadie and Robertson 1998). We, therefore, suggest that our results have clear ecological relevance under field conditions.

The reason for more hyphae in starved larvae was not investigated, but it might be related to the effect that reduced food has on the time between larval molts. Reduced food levels increase the intermolt interval (Crosskey 1990), thereby increasing the time that the hindgut is exposed to trichospores and the time before hyphae are lost as a result of shedding the hindgut cuticle at molting.

The detrimental associations previously noted between trichomycetes and their hosts (Coluzzi 1966, Dubitskii 1978, Shimada et al. 1995) have been seen as parasitic; however, we argue that more instructive descriptions are available. A parasitic relationship typically is viewed as a symbiotic association in which one member benefits, usually nutritionally, at the expense of the other member (Paracer and Ahmadjian 2000). In many cases, parasitic relationships are relatively benign (Ewald 1994) and might have little effect on the population dynamics of species (Ewald 1994, Begon

Table 4. T-tests for relative hyphal abundance of *Smittium culisetae* in the posterior colon of larval hosts (*Simulium vittatum*). Experiment number corresponds to the experiments in Table 3.

Experiment	Dosage[1]	Relative abundance of hyphae (%±SE)[2]		t value	P
		starved larvae	fed larvae		
10	4000	76.8±7.4	17.9±6.9	6.08	<0.001
11	4000	89.5±5.4	32.9±7.1	5.41	<0.001
12	4000	81.0±5.7	30.8±7.1	5.70	<0.001
15	20 000	85.8±4.7	14.7±6.3	8.48	<0.001
16	20 000	74.9±7.2	20.5±6.1	5.40	<0.001
17	20 000	74.6±7.2	26.1±7.7	4.79	<0.001

[1]Number of trichospores ml^{-1} of larval rearing water.
[2]Relative abundance of hyphae was measured following the method of McCreadie and Beard (2003). Statistical analysis was performed on arcsine-transformed data; for purposes of comparison, percentages are presented.

et al. 1996). This view of parasitism takes into account the coevolutionary relationship between the symbionts. For example, mutualistic relationships might have begun as parasitic associations, with one species trying to gain the advantage. Mutualism then can be seen as an evolutionary draw or stalemate between erstwhile antagonists (Paracer and Ahmadjian 2000). However, the 'parasitic' associations between *Smittium* and their dipteran hosts produce disease leading to death and, therefore, could be considered pathogenic (Ewald 1993, Read 1994). Although pathogens are parasites, they are a particular type of parasite, specifically one that causes disease. Furthermore, because the host is killed or sterilized (i.e. genetically terminated) at a point when reproductive success should be maximized, these infections have the same effect on a population as do predators (Begon et al. 1996). A related concept of 'internal' predation involves parasitoids, immature insects that feed internally on other insects, eventually causing the death of the host insect (Price 1997). Accordingly, we argue that trichomycetes causing death or sterility in the host can be described more accurately as pathogens or fungal parasitoids.

In summary, the symbiotic association between trichomycetes and black flies is dynamic: commensalistic when larvae are well fed, but mutualistic when larvae are starved. In the adult host, the symbiotic association is parasitic or, more accurately, it is a host-pathogen or host-parasitoid relationship. To our knowledge, the trichomycete-simuliid symbiosis is the first reported example of a relationship shifting among the three states (commensalism, mutualism, parasitism) in a single pair of associated organisms. The infection of the female ovary represents a dispersal mechanism for fungi living in flowing water (Lichtwardt 1996). Most female black flies move upstream during the egg-laying phase (Crosskey 1990), and those females with infected ovaries would transport the fungi back upstream (Labeyrie et al. 1996, Lichtwardt 1996). If the female black fly represents a significant dispersal mechanism for trichomycetes, selection not to harm the larva should be strong, underscoring the dynamic shift of symbiotic states as developmental stages of the host change.

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