Vertical Transmission of a *Nosema* sp. (Microsporida: Nosematidae) Infecting a Grasshopper, *Chorthippus curtipennis* (Orthoptera: Acrididae)

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ABSTRACT Vertical transmission studies of a *Nosema* sp. infecting a grasshopper, *Chorthippus curtipennis* (Harris), were conducted in the laboratory. Mated pairs of field-collected grasshoppers were allowed to oviposit, and the adults and progeny were subsequently examined for infection. Infection status of the parents had no effect on fecundity. A preliminary analysis of observed differences in percentage hatch was also not significant, but further analysis suggested that there were differences between mated pairs. The analysis suggested that some of the differences in hatch rate were caused by infection of the female when mated pairs were subsequently classified as having a high or low hatch rate. Vertical transmission was maternally mediated, with >70% of the progeny infected at 5 d after hatch.

KEY WORDS Microsporidia, vertical transmission, epizootiology

AN UNDESCRIBED MICROSPORIDIUM was detected in the grasshopper *Chorthippus curtipennis* (Harris) at two study sites 3 km apart at an elevation of ≈2,154 m in Madison County, MT, by McGuire & Streett (1989). They reported a relatively high prevalence of infection and evidence of infection in first nymphal instars during 1986. The host-population density was lower in 1987 with a variable prevalence of infection. The undescribed microsporidium has now been tentatively identified as a *Nosema* sp. infecting the gastric caecae, alimentary tract, and gonads of *C. curtipennis* (unpublished data).

C. curtipennis is a widespread species with a distribution encompassing the western and northern United States. Adults appear in midsummer, and oviposition occurs in mid- to late summer. At high elevations, eggs apparently require almost 2 yr to complete embryonic development before hatch. Thus, populations in consecutive seasons apparently represent distinct subpopulations of *C. curtipennis*. The parasite was present at both study sites during consecutive seasons of both high and low host-population density.

Consequently, it was concluded that a vertical transmission component must exist for the pathogen to maintain itself during successive low and high population years and because infection was reported in first nymphal instars of *C. curtipennis* (McGuire & Streett 1989). The study reported here was undertaken to investigate vertical transmission of the *Nosema* sp. in *C. curtipennis* and the effects of parental infection on host fecundity and egg viability.

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Materials and Methods

Mating Experiment. *C. curtipennis* fifth instars were field-collected, reared in the laboratory until adulthood, and separated by sex for the mating experiments. Adults were paired randomly, and allowed to mate and deposit eggs. Mated pairs were maintained in rearing tubes of sheet acetate that were 20 cm long by 9 cm in diameter. The top ends were sealed with Kerr (Kerr Glass Mfg., Los Angeles, CA) wide-mouth rings with screen inserts, and the bottom ends were inserted into styrofoam cups (12 cm diameter) that were filled with washed sand. The adults were reared in a greenhouse and fed rye and wheat bran daily. Sand was sifted daily for egg pods. Two weeks after their first egg pod was found in the rearing tube, the infection status of the adults was determined through wet-mount examinations with phase microscopy. Mated pairs were assigned to one of four combinations of healthy (H) and microsporidian-infected (I) adults (H♂ × H♀; H♂ × I♀; I♂ × I♀; I♂ × H♀). Twenty-six mated pairs were established, resulting in multiple observations (egg pods) within the four mated-pair combinations. One of the mated pairs died before laying an egg pod and was not included in the data analysis.

Table 1. Vertical transmission and effects on fecundity of the *Nosema* sp. in *Chorthippus curtipennis*

Infection status of pair[a]	No. pairs	Avg fecundity[b] ± SD	Pair hatch rate[c]		Overall hatch rate		No. of nymphs examined	Prevalence of infection[d]
			High	Low	%	No.		
H♂ × H♀	5	21.8 ± 12.8	5	0	93	101	74	20.3
I♂ × H♀	3	17.0 ± 6.2	3	0	92	47	44	18.2
H♂ × I♀	5	16.4 ± 9.9	4	1	93	76	66	77.3
I♂ × I♀	12	15.7 ± 5.0	4	8	69	131	112	72.3
Total	25		16	9				

[a] H, healthy adults; I, infected adults.
[b] Number of eggs per pair.
[c] Low hatch rate <80%; high hatch rate >80%.
[d] Percentage of nymphs infected 5 d after hatch was determined with phase microscopy.

Egg pods were stored individually in sealed vials (26 ml) with moistened vermiculite for 30 d at room temperature and then maintained at 4°C for 70 d to break egg diapause. The egg pods were returned to 30°C and examined daily for egg hatch. Progeny from individual egg pods were fed and maintained for 5 d after hatch in acetate rearing tubes, before being examined for evidence of infection. It had previously been determined that spores from vertical transmission were detected in first-instar nymphs between 3 and 5 d after hatch, but spores were not detected in inoculated nymphs until 7–9 d after inoculation (D.A.S., unpublished data). Thus, it was unlikely that cross contamination of progeny affected prevalence of infection in this study.

Differences in the total numbers of eggs oviposited (fecundity) were related to the infection status of each parent using a two-way analysis of variance (ANOVA) (Proc GLM, SAS Institute 1987) with linear comparisons (Sokal & Rohlf 1981). Disease prevalence among progeny was expressed as logit values and was weighted by the inverse of the associated variance (Neter et al. 1985). A preliminary one-way ANOVA determined that prevalence of disease did not vary between successive egg pods from the same mated pair ($P > 0.10$). An overall logit value for prevalence was then computed across all pods for each mated pair. A three-way ANOVA was used to relate prevalence to the parental infection status, the total number of eggs deposited by the female, and all interactions. In both of the preceding analyses, nonsignificant interactions ($P > 0.10$) were removed in a stepwise fashion to obtain the final model. Reductions in hatch rate were evaluated with the Maximum Likelihood (G) test (Proc Freq, SAS Institute 1985). Pairwise comparisons of the difference in hatch rates were obtained by evaluating the change in the likelihood value of the model when the two categories were combined.

Results and Discussion

Average fecundity, percentage egg hatch, and percentage of infection among progeny are presented in Table 1 for each combination of mated pairs infected with the *Nosema* sp. Infection status of the parents had no affect on the average number of eggs per mated pair ($F = 0.79$; df = 2, 22; $P = 0.464$). Neither the overall ANOVA nor any of the linear contrasts showed significance ($P > 0.35$). In contrast, Thomson (1958b) reported a significant reduction in fecundity for females of the spruce budworm, *Choristoneura fumiferana* (Clemens) infected with *Nosema fumiferanae* (Thomson).

A preliminary analysis suggested that the observed differences in percentage hatch were also not significant ($P > 0.2$); however, examination of the data indicated that this might have been a result of high variability when both parents were infected. Further analysis suggested that there were differences between mated pairs ($G = 118$, df = 24, $P < 0.001$) and that the values of percentage hatch fell into two distinct groups, high (93 to 100%) and low (42 to 77%) hatch rates, which could be separated by a cutoff value of 80%. Hatch rates from mated pairs within each group showed no differences ($G = 16.966$, df = 15, $P = 0.321$; $G = 9.194$, df = 8, $P = 0.326$ for the high and low rates, respectively).

Mated pairs were subsequently classified as low or high depending upon whether the hatch rate was above or below the 80% cutoff point. The overall analysis of these data (Table 2) suggested that the proportion of mated pairs with reduced hatch was not independent of the infection status ($G = 12.39$, df = 3, $P = 0.006$). Examination of the pairwise comparisons indicated

Table 2. Overall analysis and pairwise comparisons of hatch rate from egg pods of *Chorthippus curtipennis* as a function of infection status among parents

Source	df	G	P
Overall model	3	12.391	0.006
H♂ × H♀ vs I♂ × H♀	1	0.000	1.000
H♂ × H♀ vs H♂ × I♀	1	1.498	0.221
H♂ × H♀ vs I♂ × I♀	1	8.232	0.004
I♂ × H♀ vs H♂ × I♀	1	1.025	0.311
I♂ × H♀ vs I♂ × I♀	1	5.452	0.020
H♂ × I♀vs I♂ × I♀	1	3.228	0.072

that I♂ × I♀ mated pairs (69% hatch rate) represented the only category that was significantly different from H♂ × H♀ mated pairs (93% hatch rate, $G = 8.232$, df = 1, $P = 0.004$). The I♂ × H♀ mated pairs (92% hatch rate) were also significantly different from I♂ × I♀ mated pairs ($G = 5.452$, df = 1, $P = 0.020$), suggesting that some of the difference between the H♂ × H♀ mated pairs and the I♂ × I♀ mated pairs was caused by infection of the female. On the other hand, the hatch rate of 93% from H♂ × I♀ mated pairs was not clearly discernable from either I♂ × I♀ ($G = 3.228$, df = 1, $P = 0.072$) or I♂ × H♀ mated pairs ($G = 1.025$, df = 1, $P = 0.311$). Although it appears that infection of the female reduces the percentage hatch in a proportion of the mated pairs, it remains unclear what role infection of the male might play. The small number of observations with only one parent infected has made it impossible to assess whether reduction in hatch results from infection only of the female (at least one pair with reduced hatch occurred in each of the categories with infected females, whereas no reduction was observed in categories with healthy females) or whether infection of both parents was necessary for reduced hatch to occur. Our observation that infection of the female reduces egg hatch in a proportion of the mated pairs differed from Thomson's (1958b) report on *N. fumiferanae*-infected *C. fumiferana* females, which showed no significant difference between infection status of the parents and the egg hatch rate.

Results reported by McGuire & Streett (1989) of a 70% prevalence of infection in early instar nymphs of *C. curtipennis* during a high population year suggested some mode of vertical transmission. Diplokaryotic stages (presumably sporonts) were found in Giemsa-stained smears of embryos removed from washed eggs (unpublished data). Thus, the parasite was transovarially transmitted to the progeny. Vertical transmission for insect pathogens occurs most commonly through the female parent (Fine 1975), and this appears to be the case for the *Nosema* sp. infecting *C. curtipennis*. Vertical transmission of the parasite was maternally mediated ($F = 7.20$; df = 1, 21; $P = 0.014$; overall ANOVA, $F = 3.02$; df = 3, 21; $P = 0.053$), with over 70% of the progeny infected with the pathogen. Maternal-mediated transmission has been reported for *Nosema bombycis* Naegeli in laboratory-infected *Bombyx mori* (L.) but occurs at a higher frequency of 100% with no evidence of paternal-mediated transmission (Han & Watanabe 1988). Wilson (1982) has also reported that vertical transmission of *N. fumiferanae* in *C. fumiferana* was maternally mediated, with nearly 90% of the progeny infected with the parasite.

The potential for paternal-mediated transmission from I♂ × H♀ mated-pair combinations of *C. curtipennis* could not be established (Table 1). Indeed, 78% of the progeny from a single I♂ × H♀ mated pair were infected and contributed most of the 18% infection in the group, which led to the conclusion that infection in the parents had been misdiagnosed. Infection status of *C. curtipennis* males was not a significant factor ($F = 0.05$, df = 1, $P = 0.830$) in the statistical analysis. Infected progeny detected in control mated pairs were similarly attributed to an error in diagnosis because almost all of the infected progeny were found in a single H♂ H♂ × H♀ mated pair. When this observation was removed from these data, the prevalence of infection was reduced to 6.6%. Although paternal-mediated vertical transmission has been documented for microsporidia-infected insects, it usually occurs less frequently than maternal-mediated vertical transmission (Thomson 1958a, Kellen & Lindegren 1971). Paternal transmission of *N. fumiferanae* was observed in three of 10 *C. fumiferana* mated pairs, which led Thomson (1958a) to conclude that infected males were capable of transmitting the parasite. However, Wilson (1982) did not observe any paternal-mediated vertical transmission in infected *C. fumiferana*. Kellen & Lindegren (1971) also reported evidence of paternal transmission for *Vairimorpha plodiae* (Kellen & Lindegren) in the Indian-meal moth, *Plodia interpunctella* (Hübner), and Brooks (1968) observed stages of *Nosema heliothidis* Lutz & Splendore in the spermatogonia and spermatozoa and concluded it was likely that paternal-mediated transmission probably occurred in the corn earworm, *Helicoverpa zea* (Boddie). Thus, although paternal transmission has been reported for microsporidia, we did not conclude that paternal transmission occurred for the *Nosema* sp. infecting *C. curtipennis*.

Evidence of spores within 3 to 5 d after hatch and the report by McGuire & Streett (1989) of spores detected in first-instar *C. curtipennis* in the field suggested a relatively rapid development of the parasite to the spore stage. This observation and the successive low and high population years of *C. curtipennis* indicated the presence of some mode of vertical transmission for the parasite. Results of this study confirm that maternal-mediated vertical transmission occurs for the *Nosema* sp. infecting *C. curtipennis*. Although the parasite does not significantly affect fecundity, a significant reduction in hatch rate was observed for eggs from some infected females.

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References Cited

Brooks, W. M. 1968. Transovarian transmission of *Nosema heliothidis* in the corn earworm, *Heliothis zea*. J. Invertebr. Pathol. 11: 511–512.

Fine, P.E.M. 1975. Vectors and vertical transmission: an epidemiologic perspective. Ann. N.Y. Acad. Sci. 266: 173–194.

Han, M.-S. & H. Watanabe. 1988. Transovarial transmission of two microsporidia in the silkworm, *Bombyx mori*, and disease occurrence in the progeny population. J. Invertebr. Pathol. 51: 41–45.

Kellen, W. R. & J. E. Lindegren. 1971. Modes of transmission of *Nosema plodiae* Kellen and Lindegren, a pathogen of *Plodia interpunctella* (Hübner). J. Stored Prod. Res. 7: 31–34.

McGuire, M. R. & D. A. Streett. 1989. Epizootiology of an undescribed microsporidium in populations of *Chorthippus curtipennis* (Orthoptera: Acrididae). J. Invertebr. Pathol. 54: 272–274.

Neter, J., W. Wasserman & M. H. Kutner. 1985. Applied linear statistical models, 2nd ed. Richard D. Irwin, IL.

SAS Institute. 1985. SAS guide for personal computers, version 6 ed. Cary, NC.

1987. SAS/STAT guide for personal computers, version 6 ed. Cary, NC.

Sokal, R. S. & F. J. Rohlf. 1981. Biometry, 2nd ed. Freeman, New York.

Thomson, H. M. 1958a. Some aspects of the epidemiology of a microsporidian parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). Can. J. Zool. 36: 309–316.

1958b. The effect of a microsporidian parasite on the development, reproduction, and mortality of the spruce budworm, *Choristoneura fumiferana* (Clem.). Can. J. Zool. 36: 499–511.

Wilson, G. G. 1982. Transmission of *Nosema fumiferanae* (Microsporida) to its host *Choristoneura fumiferana* (Clem.). Z. Parasitenkd. 68: 47–51.

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