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Life History Differences Between Two Species of *Gregarina* in *Tenebrio molitor* Larva

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LIFE HISTORY DIFFERENCES BETWEEN TWO SPECIES OF *GREGARINA* IN *TENEBRIO MOLITOR* LARVAE

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ABSTRACT: The life histories of 2 species of *Gregarina*, protozoans parasitizing *Tenebrio molitor*, were compared. *Gregarina cuneata* occurred primarily in larger larvae, whereas *Gregarina steini* was distributed evenly throughout all sizes of larvae. As larval size increased, an increase in prevalence of *G. cuneata* preceded a more substantial increase in parasite relative density. Sample variance/relative density ratios of the 2 parasite species also differed. When host larvae were isolated in a sterile bran medium, the prevalence of *G. cuneata* decreased, whereas that of *G. steini* remained relatively high. Trophozoites of *G. cuneata* were found predominantly in a paired condition, whereas trophozoites of *G. steini* typically were solitary.

Gregarina cuneata Stein, 1848, and *Gregarina steini* Berndt, 1902, are intestinal parasites of the grain beetle *Tenebrio molitor* L. Both have been reported from *T. molitor* in nature (Lipa, 1967; Harry, 1973) and often are present in laboratory beetle colonies. The 2 gregarine species constitute a system for comparing how congeneric parasites utilize a single host species. For example, *G. cuneata* and *G. steini* exhibit noninteractive site specificity within the midgut of *T. molitor* larvae (Ruhnke and Janovy, 1989). The cooccurrence of these 2 gregarine species allowed for the formulation of several questions concerning their life histories: (1) Do the patterns of relative density, aggregation (variance/relative density), and gregarine gametocyst formation differ between the 2 species of *Gregarina* with respect to the size of the larval host? (2) Does prevalence of infection with either species of *Gregarina* vary with the size of the larval host? (3) Do the 2 gregarine species exhibit intraspecific trophozoite pairing at similar times within the host midgut? (4) What patterns of prevalence and gametocyst formation are manifest when infected beetle larvae are isolated in a sterile medium and not allowed to encounter additional infective stages of the gregarines?

These questions were designed to provide a detailed account of the life histories of *G. cuneata* and *G. steini* and to allow for the understanding of how these species maintain themselves in their common host species. Answers to these questions also provide an initial comparative basis for studies of how such host–parasite systems have evolved.

MATERIALS AND METHODS

Three laboratory beetle colonies were kept in aquaria and maintained on wheat bran and potato slices. These 3 colonies were considered replicates for statistical analyses. For the purpose of this study, the host larvae were divided into 4 size-classes: 6–10 mm, 11–15 mm, 16–20 mm, and >20 mm. The parasite species were identified as *G. cuneata* and *G. steini* using the original description by Berndt (1902) and characters and figures given in monographs by Lipa (1967) and Watson (1916).

Two hundred sixty-seven beetle larvae were measured and cut in half with forceps. These halves were moved to opposite ends of a glass slide. Midgut sections were pulled away from the surrounding exoskeleton, cleaned of fat, and opened longitudinally with a fine probe. Intestinal contents were examined by light microscopy at 100×. Data were recorded from these dissections as follows: species of parasite, number of gregarine trophozoites in the single and paired condition, and number of gregarine gametocysts. Grand means of parasite relative density, their associated standard deviations, and pooled prevalences were calculated within size-classes for the 2 gregarine species.

Two hundred beetle larvae from the same 3 colonies were measured and placed singly in small petri dishes. These larvae were allowed to defecate for 48 hr. The number of gametocysts shed by each larva in the 48-hr period was recorded for both species of *Gregarina*. The gametocysts of *G. cuneata* were large, whitish, and spherical, whereas those of *G. steini* were smaller, ovoid, and somewhat golden in color. Grand means of gregarine gametocysts shed and their standard deviations were calculated.

Data from these experiments were used to test the null hypotheses: Relative density and the rate of gametocyst shedding are independent of host size in both parasite species (see question 1). Analysis of variance (ANOVA) (Anonymous, 1985) was performed on the numerical ranks of parasite infrapopulations and gametocyst formation for each gregarine species within each sample colony. Infrapopulational values were ranked numerically to normalize the data. In the cases where null hypotheses were rejected, Duncan's mul-

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TABLE I. A summary of the parasite population distribution patterns in the 4 size-classes of *Tenebrio molitor* larvae.

Larval size-class (length in mm)	Relative density grand mean	Standard deviation	Pooled prevalence	n
Gregarina cuneata				
6–10	2.9	6.1	0.38	84
11–15	54.2	160.9	0.58	65
16–20	132.1	218.3	0.70	57
>20	369.2	598.8	0.72	61
Gregarina steini				
6–10	15.2	55.3	0.38	84
11–15	22.2	148.7	0.49	65
16–20	49.5	133.3	0.63	57
>20	42.1	81.8	0.51	61

tiple range test (DMRT) was used to determine significant differences between groups. Differences in parasite intraspecific prevalence were tested by using a rolling 2-by-2 contingency table analysis. Chi-square values were used to test the null hypothesis of no intraspecific difference in prevalence within the sample colony between larval size-classes. Percentages of single and paired (=associated) trophozoites in infected *T. molitor* larvae were calculated for each of the 2 gregarine species, using data collected from sample colonies 2 and 3.

In the isolation studies, beetle larvae were measured for placement into the 3 smallest size groups: 6–10 mm, 11–15 mm, and 16–20 mm. These larvae were maintained in autoclaved bran in finger bowls. They were fed washed slices of potato and the autoclaved bran was replaced weekly to prevent autoinfection of larvae. Nine larvae/size-class/week were isolated singly in small petri dishes. Forty-eight hours later the feces of the isolated larvae were checked for the presence of gametocysts. The length of each larva and the number of gametocysts shed were recorded. The beetle larvae then were dissected to detect the presence of 1 or both species of *Gregarina*. Prevalence of infection and prevalence of cyst shedding were recorded each week for 4 wk. This procedure was repeated 6 times with 108 larvae in each replicate. Because 5 larvae died, only 643 were dissected during this part of the study.

Terminology here is consistent with that given by Margolis et al. (1982). Variance/relative density is a measure of aggregation.

RESULTS

Gregarina cuneata exhibited an increase in relative density as the size of the larval host increased (Table I). In all 3 colonies, ANOVA on numerically ranked data indicated a significant difference between larval size-classes. DMRT revealed that the mean numerical ranks of the largest larval size-class were consistently greater than the smaller 2. Relative density of *G. steini* did not increase as the size of the larval host increased (Table I), and no statistical difference was found between groups with ANOVA. Variance/relative density ratios for *G. cuneata* ranged from 6.8 to 1,321.0 and were greater than ratios calculated for *G. steini*, with ratios ranging from 2.6 to 537.0 (Table II).

TABLE II. Sample variance/relative density ratios for the 2 species of *Gregarina* in *Tenebrio molitor* larvae.

Larval size-class (length in mm)	Sample variance/relative density ratios		
	Colony 1	Colony 2	Colony 3
G. cuneata			
6–10	14.3	13.5	6.8
11–15	638.0	38.4	148.0
16–20	211.5	421.5	505.6
>20	1,321.0	359.0	940.0
G. steini			
6–10	2.6	86.9	285.5
11–15	68.0	289.6	504.9
16–20	263.0	62.4	537.0
>20	59.7	210.0	170.5

Intraspecific rates of gametocyst shedding also differed between the 2 species of *Gregarina*. Gametocysts of *G. cuneata* were released predominantly from the largest size-class of beetle larvae (Table III). This difference was significant statistically in 2 of the 3 sample colonies. DMRT indicated that in both cases the largest larvae shed more gametocysts than smaller size groups, but the difference was significant only in colony 3. A slight increase in gametocyst shedding from larger larvae was observed for *G. steini* (Table III), but these differences were not significant statistically.

Significant differences in prevalence (chi-square values 5.13 and 4.21 and Table I) were found for *G. cuneata* infections between the 6–10-mm and 11–15-mm host size-classes in colonies 1 and 3. No significant difference in parasite prev-

TABLE III. Summary of gametocyst shedding data for 2 species of *Gregarina* in *Tenebrio molitor* larvae.

Larval size-class (length in mm)	Grand mean of gametocysts shed/ larvae/48 hr	Standard deviation	n
G. cuneata			
6–10	0.08	0.45	50
11–15	0.14	0.45	50
16–20	3.32	11.45	50
>20	29.4	58.0	60
G. steini			
6–10	0.22	0.63	50
11–15	0.24	0.85	50
16–20	0.44	1.66	50
>20	1.5	3.87	60

TABLE IV. The percentage of single and paired trophozoites of *Gregarina cuneata* and *Gregarina steini.*

Larval size-class (length in mm)	Percentages of trophozoites	
	Single	Paired
G. cuneata		
6–10	35	65
11–15	21	75
16–20	11	89
>20	87	13
G. steini		
6–10	87	13
11–15	76	24
16–20	77	23
>20	80	20

alence was found between larval size groups infected with *G. steini.*

Trophozoites of *G. cuneata* were found primarily paired in *T. molitor* larvae, whereas trophozoites of *G. steini* generally were present in a solitary condition (Table IV).

A summary of prevalence of infection and gametocyst shedding for the 2 species of *Gregarina* in isolation is given in Table V. The prevalence of *G. cuneata* decreased as the number of weeks in isolation increased. Initial prevalence of this species was lowest in the 6–10-mm larval size-class and highest in the 16–20-mm size-class. With the exception of 1 host individual, gregarine gametocysts were shed only by larvae in the size range of 16–20 mm. As time in isolation increased, the prevalence of *G. steini* did not decrease in the same fashion as *G. cuneata.* The species was found in all larval size-classes during the experiment, and gametocysts of *G. steini* were shed by larvae in all size-classes (Table V).

TABLE V. Summary of parasite prevalences of infection and gametocyst shedding in larval *Tenebrio molitor* during the isolation experiments.

Category*	Larval size-class (length in mm)	Prevalence (by week)			
		1	2	3	4
Gregarina cuneata					
A	6–10	0.30	0.24	0.04	0.05
B		0	0	0	0
A	11–15	0.41	0.13	0.06	0.06
B		0.02	0	0	0
A	16–20	0.67	0.28	0.17	0.17
B		0.17	0.06	0.04	0
Gregarina steini					
A	6–10	0.39	0.54	0.65	0.50
B		0.03	0.15	0.19	0.13
A	11–15	0.57	0.57	0.48	0.38
B		0.11	0.06	0.02	0.06
A	16–20	0.65	0.61	0.37	0.44
B		0.15	0.13	0.10	0.15

* A is the prevalence of infection, and B is the prevalence of gametocyst shedding.

DISCUSSION

The 2 congeneric parasite species are different with respect to their population biology and life history in a single host species. *Gregarina cuneata* is associated strongly with larger *T. molitor* larvae, as measured by the variance/relative density ratio. *Gregarina steini* is distributed uniformly throughout all host size-classes. Furthermore, gametocyst shedding in *G. cuneata* is an attribute of infections in larger larvae, whereas in *G. steini* it is independent of host size. Patterns of parasite prevalence between the size-classes of host also differed interspecifically.

An interesting finding concerning the population biology of these 2 gregarine species was their different patterns of aggregation within various size-classes of their insect host; *G. cuneata* was highly aggregated in larger host larvae (Table II). This was not observed in *G. steini.* The basis for the difference in aggregation (as measured by the variance/relative density ratio) of the 2 gregarine species may be in the different scheduling of infective stages into the environment (Table III) and their resulting spatial distribution. Gametocyst formation of *G. cuneata* is almost exclusively a function of larger beetle larvae. In the experimental colonies larger larvae routinely were observed feeding on potato at the top of the bran. Smaller larvae were found throughout the wheat bran. These observations suggest that *T. molitor* larvae segregate by size within the experimental colonies. Such a factor could have produced infective foci of *G. cuneata* at the top of the colonies. Larger larvae feeding and defecating there would then become heavily infected and subsequently contribute to infective foci by depositing gametocysts. Gametocysts of *G. steini* are released at an equal rate by all size-classes of beetle larvae and are available in all regions of the experimental colonies. Aggregation of this gregarine species would not be predicted, even if the larger host larvae spatially segregate to feed. The results of this study are consistent with those of Keymer and Anderson (1979) who demonstrated experimentally that the rate and distribution of parasite infective stages influenced patterns of relative density and aggregation of the parasite species. This study also confirms the theoretical prediction made by Janovy and Kutish (1988) that variance/relative density ratios should

change as the number, rate, and distribution of parasite infective stages are manipulated.

The difference in the rate of gregarine pairing of the 2 species suggests that the pairing behavior may be useful in the taxonomy of other species of *Gregarina*. Different modes of trophozoite pairing already are used in the classification of the eugregarines. For example, parasites of the genus *Stylocephalus* Ellis, 1912, are solitary throughout their trophic phase of the life cycle and pair head-to-head immediately before encystment (Lee et al., 1985; and personal observation). Data of the type reported here would allow eugregarine taxonomists not only to diagnose taxa better, but would also add specific information on the nature of life cycle differences between gregarine species.

The loss of *G. cuneata* when its larval host is isolated in a sterile medium is somewhat difficult to explain. Harry (1973) reported observations on the life histories of *Gregarina polymorpha* (Hammerschmidt, 1838) Stein, 1848, *G. cuneata, G. steini,* and *Steina ovalis* (Stein, 1848) Leger and Duboscq, 1904, from wild populations of *T. molitor* larvae. He found that as the temperature dropped below 10 C, beetle larvae ceased feeding. Infections of *G. cuneata* and *G. polymorpha* were lost over the winter whereas the other 2 species persisted. Although our larval isolation experiments were conducted at room temperature, it is still possible that a change in habitat (from the stock to the sterile bran) stressed the larvae, affecting them in a fashion similar to that of temperature in Harry's (1973) study.

It is clear from the present study that although these 2 species presumably share a common phylogenetic heritage, as well as the same life cycle (both are direct), the details of their life histories are different. Studies of other species of gregarines could reveal whether 1 or both of the 2 gregarine life history patterns reported here are unique to these 2 species or are generalized patterns shared by other species. Such studies would help to build hypotheses on the evolution of gregarine life histories.

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