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# HOST STADIUM SPECIFICITY IN THE GREGARINE ASSEMBLAGE PARASITIZING *TENEBRIO MOLITOR*

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ABSTRACT: Reciprocal cross-stadia experimental infections were used to demonstrate stadium specificity within the gregarine assemblage parasitizing *Tenebrio molitor*, the yellow mealworm. *Gregarina cuneata, Gregarina polymorpha*, and *Gregarina steini* are characteristic parasites of larval *T. molitor. Gregarina niphandrodes* is a characteristic parasite of adult *T. molitor*. Experimental infections were produced in all homologous host–parasite combinations. No infection was produced in heterologous or cross-stadia combinations. This study introduces the concept of separate, distinct parasite niches corresponding to separate life cycle stages and established by known, predictable life cycle events within a single host species.

Protozoans of the genus *Gregarina* are amerogonous, stenoxenous, apicomplexan parasites of the insect midgut (Levine, 1985). *Gregarina* species have a direct life cycle that produces oocysts resistant to a wide range of environmental conditions (MacDougall, 1942; Brooks, 1974; Patil et al., 1983). Transmission is completed through accidental ingestion of infective oocysts. There is no report of autoinfection. Allegre (1948) and Levine (1985) provided excellent reviews of the life cycle.

Although the taxonomic literature of the group assumes a high degree of specificity, the nature and extent of host specificity within the genus *Gregarina* is unclear. In the most recent review of the Septatorina, Levine (1979) noted the apparent homoxenous nature of the genus and justified the use of host species as a taxonomic character because specificity recognized failure of transmission between host and nonhost species.

There have been limited attempts to produce laboratory infections using gregarines. Allegre (1948) and Tronchin et al. (1986) produced homologous experimental infections using *Gregarina rigida* (Hall, 1907) Ellis, 1913, in *Melanoplus differentialis* and *Gregarina blaberae* Frenzel, 1892, in *Blaberus craniifer*, respectively. Patil et al. (1985) addressed the question of eugregarine host specificity using 9 species of septate gregarines in the genera *Stylocephalus, Xiphocephalus*, and *Cryptocephalus* in cross-infection experiments with 8 species of tenebrionid beetles. Although exsporulation was reported in 5 heterologous host–parasite combinations, parasite establishment and growth was observed only in homologous combinations.

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Anecdotal postmortem observations from *Tenebrio molitor* stock colonies maintained at the University of Nebraska in Lincoln indicate a distinct niche separation in the associated parasite assemblage. *Gregarina cuneata* Stein, 1848, *Gregarina polymorpha* (Hammerschmidt, 1838) Stein, 1848, and *Gregarina steini* Berndt, 1902, are characteristic parasites of larval *T. molitor*; however, they do not appear to infect adult *T. molitor. Gregarina niphandrodes* Clopton, Percival, and Janovy, 1991, is a characteristic parasite of adult *T. molitor*; however, it does not appear to infect larval forms of the same host species. The homoxenous consensus reflected by the literature coupled with these anecdotal observations suggests that gregarine specificity may extend to metamorphic divisions within a single species. This study uses cross-stadia experimental infections to address the question of whether members of the gregarine assemblage infecting *T. molitor* are stadium specific.

## MATERIALS AND METHODS

The adults and larvae of *T. molitor* were used as hosts. *Tenebrio molitor* was maintained in wheat bran, with potatoes, in tubs covered with burlap, and moistened at least 3 times a week. Four gregarine species were manipulated: *G. cuneata, G. polymorpha*, and *G. steini* from *T. molitor* larvae, and *G. niphandrodes* from *T. molitor* adults.

Larval *T. molitor* often are infected with multiple gregarine species concurrently; however, the gametocysts of multiple species infections were sorted according to parasite species using gametocyst structure. The gametocysts of *G. cuneata* are approximately 240 μm in diameter and appear in grapelike clusters, whereas the gametocysts of *G. polymorpha* and *G. steini* appear singly (Mackinnon and Hawes, 1961). The gametocysts of *G. polymorpha* are white and measure approximately 190–200 μm in diameter. In contrast, the gametocysts of *G. steini* are amber to dark brown and ovoid with axial measurements rarely exceeding 100 × 160 μm (Mackinnon and Hawes, 1961). Ga-

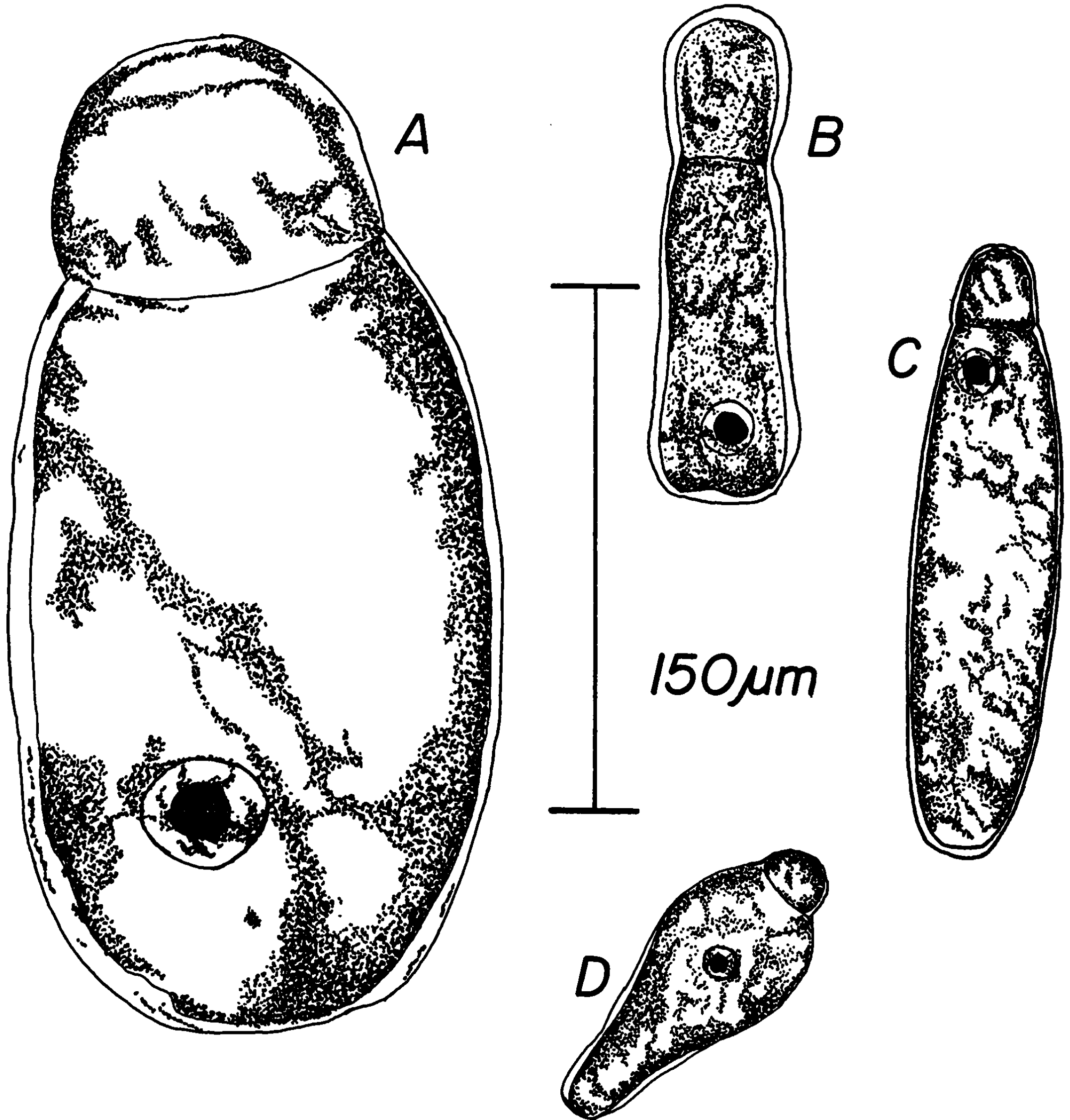


FIGURE 1. Comparison of the relative size and structure of 4 species of *Gregarina* in *Tenebrio molitor*. **A.** *Gregarina niphandrodes*, 10 days postinfection (PI). **B.** *Gregarina cuneata*, 10 days PI. **C.** *Gregarina polymorpha*, 3 days PI. **D.** *Gregarina steini*, 3 days PI.

metocysts were collected from both adult and larval *T. molitor* by placing 20–50 insects in a plastic shoebox with moist paper towels over night. Feces were examined the next morning. Gametocysts were freed by softening frass in basic saline solution and then were sorted according to parasite species and pipetted to 6-mm circles of black construction paper. The black background improved visibility of oocyst chains. The paper circles were placed in the bottom of small petri dishes that in turn were placed in larger, filter paper-lined glass culture dishes. The filter paper was kept slightly moist until dehiscence and the appearance of spore chains. Gametocysts were kept in separate dishes according to parasite species. After spore chains were shed, no water was added to the storage dishes.

Adult and larval *T. molitor* were sterilized (of gregarines) by incubation at 36–37 C for 5 days (MacDougall, 1942). This treatment successfully eliminated existing gregarine infections.

Sterilized adult and larval *T. molitor* were divided, respectively, into 3 groups of 5–7 animals each: a time zero control ($T_0C$), an ending time control ($T_tC$), and an experimentally infected group ($T_tE$). $T_0C$ animals were killed and examined for parasites at the onset of the experiment to ensure the efficacy of the sterilization process. $T_tE$ animals were allowed to feed for 24 hr on

TABLE I. Mean number of parasites/beetle for reciprocal cross stadia experimental infections among the gregarine parasites of *Tenebrio molitor*.*

| | $T_0$ control† | | $T_t$ control† | | $T_t$ experimental† | |
|---|---|---|---|---|---|---|
| | Adults | Larvae | Adults | Larvae | Adults | Larvae |
| *Gregarina niphandrodes* | 0.0 | 0.0 | 0.0 | 0.0 | 37.1 | 0.0 |
| *Gregarina cuneata* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 12.9 |
| *Gregarina polymorpha* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 23.0 |
| *Gregarina steini* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 30.5 |

* n = 5 beetles in each of 3 replications.

† $T_0$, control group examined at beginning of the experimental infection; $T_t$, control group examined at the termination of the experimental infection; $T_t$, experimental group examined at the termination of the experimental infection.

ca. 0.125-cm³ potato plugs contaminated with oocysts. $T_t$C animals were fed on clean potato plugs during the same period. $T_t$C and $T_t$E animals were held in plastic shoeboxes with ca. 30 ml of flour and a moist paper towel in a 22-C incubator for development of the infection. Hosts infected with each parasite species were kept separate from other experimental groups. $T_t$C and $T_t$E animals were killed and examined for parasites either 3, 5, or 10 days postinfection. All replications of the *G. cuneata* versus *G. niphandrodes* reciprocal cross infections were carried to 10 days postinfection. The first replications of the *G. polymorpha* and *G. steini* versus *G. niphandrodes* reciprocal cross infections were carried to 5 days postinfection, whereas the second and third replications were carried to 3 days postinfection. On postmortem examination of infected animals, the number of parasites present and their sizes were recorded. The relative size and structure of each species as observed postmortem are depicted in Figure 1. The experimental design was replicated 3 times for each parasite species examined.

Reciprocal cross infections involved *G. cuneata, G. niphandrodes, G. polymorpha,* and *G. steini* in both adult and larval *T. molitor* (i.e., in both homologous and heterologous host life cycle stages) as a means of ensuring infectivity of oocysts for the homologous life cycle stage and ensuring ingestion of oocysts of both homologous and heterologous hosts.

Terminology used in this paper is consistent with that of Levine (1971).

## RESULTS

The mean numbers of parasites/host for all experimental infections are presented in Table I. *Gregarina niphandrodes,* characteristically a parasite of adult *T. molitor,* established in experimental adult beetles, but not in experimental larval beetles. *Gregarina cuneata, G. polymorpha,* and *G. steini,* all characteristically parasites of larval hosts, established in experimental larval beetles, but not in experimental adult beetles. In summary, for all experiments and replications, experimental infections were produced in homologous but not heterologous beetles. No infection was recorded in the control groups. Morphological measurements of parasites within each infection are presented in Table II. In all experiments and replications, the morphological variability among parasites within an experimental group was low, regardless of the age of the infection.

## DISCUSSION

The reciprocal cross-stadia experimental infection data demonstrate a clear separation of the host life cycle into 2 parasite niches by a known and predictable host life cycle event. The division is recognized by all known members of the host's gregarine assemblage. The changes associated with holometabolic insect development make the adult gut unacceptable to gregarines that are characteristically parasites of the larval gut. In a like manner, parasites characteristic of the adult gut do not establish and grow in the larval gut. The association between insects and gregarines is regarded as ancient. Stadium-specificity in a holometabolic insect host such as *T. molitor* suggests that the relationship predates the rise of the holometabolic life style. The paucity of species parasitizing adult forms in comparison to the larval forms suggests that the relationship between *T. molitor* and *G. niphandrodes* may represent a secondary host capture event in combination with a speciation event.

Although adult and larval *T. molitor* seem to occupy different physical strata in a laboratory colony, the parasitic niche separation does not appear to be a function of nonoverlapping host distributions. The inability of the members of the *T. molitor* gregarine complex to establish when artificially introduced into a heterologous host stadium suggests that stadium specificity is the result of adaptation and phylogenetic history, not host escape in time or space; i.e., it is an evolutionary and not an ecological phenomenon.

The morphological data demonstrate low variability in the growth rate of gregarines within a single experimental animal. These data provide

TABLE II. Morphometric data from 4 species of *Gregarina* in *Tenebrio molitor*.

| Parameter* | *G. niphandrodes*† (n = 35) | *G. cuneata* (n = 60) | *G. polymorpha* (n = 42) | *G. steini* (n = 40) |
|---|---|---|---|---|
| Length of protomerite | 67.8 ± 15.6 (38.4–105.6) | 43.8 ± 11.5 (19.2–76.8) | 38.0 ± 5.6 (28.8–50.6) | 14.1 ± 5.6 (7.2–33.0) |
| Width of protomerite | 87.2 ± 11.8 (67.2–105.6) | 41.9 ± 8.9 (19.2–57.6) | 37.0 ± 6.3 (24.2–48.0) | 23.8 ± 5.8 (14.4–39.6) |
| Length of deutomerite | 213.2 ± 34.1 (124.8–259.2) | 117.6 ± 29.7 (57.6–192.0) | 141.1 ± 56.4 (76.8–96.0) | 95.4 ± 28.1 (67.2–154.0) |
| Width of deutomerite | 126.2 ± 18.8 (76.8–163.2) | 49.1 ± 15.9 (19.2–86.4) | 75.2 ± 13.0 (46.2–96.0) | 39.9 ± 10.1 (24.0–63.8) |
| Total length | 280.9 ± 39.0 (163.2–355.2) | 161.4 ± 37.9 (76.8–249.6) | 179.0 ± 56.7 (105.6–347.6) | 109.5 ± 33.6 (76.8–187.0) |

* Values are means ±SD with ranges in parentheses of beetles from 3 experimental infections.
† Infections in adult beetles; all other infections were in larval beetles.

additional evidence that these parasites were members of a single cohort experimentally introduced into the host. These measurements were taken from immature gregarines and are much smaller than those reported for mature gamonts by Clopton et al. (1991). These observations suggest that consistent gregarine growth curves can be established.


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