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Host Specificity of *Gregarina blattarum* von Siebold, 1839 (Apicomplexa: Eugregarinida) among Five Species of Domiciliary Cockroaches

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The host specificity of *Gregarina blattarum* was evaluated among five species of domiciliary cockroaches: *Blatella germanica, Supella longipalpa, Blatta orientalis, Periplaneta americana,* and *Periplaneta fuliginosa.* Third- and fourth-instar nymphs were allowed to feed on crushed dog kibble contaminated with *G. blattarum* oocysts. Cockroaches were killed 8 days postinfection and examined for parasites. *Gregarina blattarum* infections were observed in all experimentally infected replications of *B. germanica.* No infection was observed in an experimentally infected replication of *S. longipalpa, B. orientalis, P. americana,* or *P. fuliginosa,* nor was an infection observed in a time zero or ending time control group. *In vitro* excystation assays using extracts of host gut homogenates demonstrate that *G. blattarum* sporozoites successfully excyst and begin the life cycle in all five cockroach species tested. No excystation was observed in neutral buffered saline controls. These data suggest that *G. blattarum* comprises a complex of cryptic species marked by narrow host utilization rather than a single species parasitizing a broad array of cockroach taxa.

KEY WORDS: ***Gregarina blattarum*; *Blattella germanica*; *Supella longipalpa*; *Blatta orientalis*; *Periplaneta americana*; *Periplaneta fuliginosa*; host specificity; infection, parasitic; excystation, development.**

INTRODUCTION

Eugregarinida (Protista: Apicomplexa) are obligate enteric parasites infecting a taxonomically diverse array of invertebrate hosts including annelids, crustaceans, echinoderms, arachnids, pelagic tunicates, and insects (Grassé, 1953). The literature suggests that rigid host specificity is a general feature of the gregarines. However, the general trend may be an artifact of incomplete survey: nearly 80% of known gregarine species have not been reported since their original description (Levine, 1988).

Strict host specificity has been empirically demonstrated in several gregarine systems. *Ascogregarina culicis* (=*Lankesteria culicis*) was once believed to parasitize at least 9 mosquito species, although repeated attempts to transmit parasites from 1 host species to another failed (see Lien and Levine, 1980 and Walsh and Olson, 1976 for reviews of *Ascogregarina culicis* cross-transmission studies). Additional morphological and cross-transmission studies confirmed that *Ascogregarina* was a complex rather than a single species (e.g., Vávra, 1969). As a result, the *Ascogregarina* complex now comprises 14 distinct species (Levine, 1988).

In some systems, host specificity extends beyond species differences to recognize ontogenetic stages of a single host species. Host stadium specificity has been demonstrated within the gregarine assemblage parasitizing *Tenebrio molitor. Gregarina polymorpha, Gregarina cuneata,* and *Gregarina steini* are restricted to larval *T. molitor* and *Gregarina niphandrodes* is restricted to adult beetles (Clopton *et al.,* 1992).

In contrast, other studies indicate some gregarine species may be more catholic in their host associations. Corbel (1968) studied the cross-infectivity of 6 gregarine species among 24 orthopteran species and concluded that, among gregarines infecting orthopterans, strict specificity was apparent only at the level of host superfamily. He concluded that strict parasitological specificity seems be rare at the host species level, at least among gregarines parasitizing orthopterans (Corbel, 1968).

Unique host associations have often been used as cardinal species characters within Eugregarinida. In some cases, gregarine populations have been recognized as new species solely because they represent unique host–parasite combinations. Levine (1988) noted the fallacious nature of the "different host, dif-

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ferent parasite" idea. He concluded that host association may be a valid indicator of species status, but the empirical and survey data are not sufficient to extend the concept to all cases. This observation suggests that cases of broad host utilization are also suspect. The apparent morphological simplicity of the gregarines may mask a variety of unrecognized cryptic species. Gregarines commonly associated with domiciliary cockroaches may be a case in point.

Gregarina blattarum was originally described from wild populations of *Blatta orientalis* collected in Germany (von Siebold, 1839). *Gregarina blattarum* has since been reported from wild-type and laboratory populations of *B. orientalis, Periplaneta americana, Periplaneta discoidales, Blattella germanica, Parcoblatta pennsylvanica,* and *Blaberus craniifer* in the United States (Crawley, 1903; Ellis, 1913; Hall, 1907; Lankester, 1863; Leidy, 1853; Seamans, 1943; Sprague, 1941), Brazil (de Magalhaes, 1900), France (Schneider, 1875), Germany (Kölliker, 1848; Stein, 1848; von Frantzius, 1848), Poland (Lipa, 1967), and Japan (Abe, 1977; Abe and Tsuchiya, 1980; Hashimoto and Abe, 1978; Hoshide *et al.,* 1993; Obata, 1953; Uemura and Abe, 1977) and is probably the most widely reported of known gregarine species. In each case, available morphological data from local parasite populations are consistent with that of *G. blattarum,* but the host associations have never been evaluated experimentally.

Gregarina blattarum is a ubiquitous parasite of *B. germanica* in laboratory colonies maintained in the Center for Urban Entomology, Texas A&M University. The purpose of this study was to evaluate the host specificity of this *G. blattarum* strain among the following five species of domiciliary cockroaches: *B. germanica, Supella longipalpa, B. orientalis, P. americana,* and *Periplaneta fuliginosa.* The study concerns two key life cycle events: excystation of dormant oocysts (life cycle initiation) and establishment (infection and development). Terms used for gregarine ontogentic stages are consistent with Levine (1971).

MATERIALS AND METHODS

Host and Parasite Culture

Wild-type colonies of *B. germanica, S. longipalga, B. orientalis, P. americana,* and *P. fuliginosa* were established using mixed strains collected in Houston, Temple, and College Station, Texas. Separate colony containers were used for each cockroach species. *Gregarina blattarum* populations were maintained *in vivo* with isolated *B. germanica* colonies. All colonies were incubated at 25°C and were provided with dog kibble, rat blocks, and water *ad libitum.* Ootheca were collected prior to eclosion and held individually in 60-mm petri dishes. Cockroach nymphs were collected on eclosion, transferred to pint mason jars, and maintained on dog kibble and water. These synchronized colonies provided gregarine-free nymphs of known age for experimental use.

Inoculum

Gametocysts were collected from adult and nymphal *G. blattarum* by placing 20–30 insects in a 100-mm plastic petri dish for 6 hr. The insects were removed and the collected feces examined. Gametocysts were freed by softening frass in neutral buffered saline solution, surface sterilized in 0.01% neutral buffered formalin, and pipetted in lots of 10 gametocysts onto 6-mm disks of black construction paper. Disks with gametocysts were placed in the bottom of small glass petri dishes that were in turn placed in a plastic shoebox lined with about 4 cm of damp sand and incubated at 25°C. Gametocysts matured and dehisced within 72 hr, releasing oocyst chains that were used for experimental inoculum.

Cross-Infection Protocol

Pair-wise experimental cross-infections were conducted using *B. germanica* (control species) and 1 other experimental cockroach species (experimental species). Gregarine-free cockroachs of both control and experimental species were divided, respectively, into 3 groups of 5 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 gregarine infection at the onset of the experiment to ensure uninfected experimental animals. T_tE animals were allowed to feed for 24 hr on crushed dog kibble contaminated with gregarine oocysts. T_tC animals were fed on uncontaminated dog kibble during the same period. T_tE and T_tC groups were transferred to separate 100-mm plastic petri dishes with uncontaminated dog kibble and a moist dental roll, and placed in a 25°C incubator for infections to develop. T_tE and T_tC animals were killed 8 days postinfection, examined for parasites, and the number of gregarines present was recorded. The experimental design was replicated six times for each experimental host species.

Preparation of Host Gut Tissue Extracts and Excystation Bioassay Protocol

In vitro excystation studies were conducted using a bioassay modified from Hoshide *et al.* (1993). Extracts of host gut tissue homogenates were prepared as follows. Cockroaches were dissected in neutral buffered saline (*P. americana* heart saline [Ludwig *et al.,* 1957]). The foregut, midgut, salivary glands, and salivary sac were removed from each cockroach and stored in neutral buffered saline on ice until approximately 1 ml of tissue had been collected. The collected tissue was mac-

erated in 70 μl of neutral buffered saline for 1 min with a tissue grinder and the resulting homogenate cleared of large cellular debris by centrifugation for 5 min at 3732*g*. Supernatant fluid was collected and filtered through a 0.45-μm acetate filter, producing an extract free of cellular debris and microorganisms. In the *in vitro* excystation studies, 3 μl of host tissue extract was placed on a 10×10-mm clean glass coverslip and approximately 1000 oocysts were added. A wet mount was prepared, sealed with paraffin oil, and examined under phase microscopy for evidence of sporozoite activation and excystation. *Gregarina blattarum* oocysts were tested for excystation in individual homogenates of *B. germanica, S. longipalpa, B. orientalis, P. americana,* and *P. fuliginosa* gut tissues. Excystation bioassays were replicated six times for each cockroach species. Preparations using neutral buffered saline in lieu of gut extract served as controls.

RESULTS

The mean numbers of parasites per host for all experimental infections are presented in Table 1. No infection was observed in a T_0C or T_tC group. No infection was observed in a T_tE *S. longipalpa, B. orientalis, P. americana,* or *P. fuliginosa* group. However, the *G. blattarum* strain isolated from *B. germanica* established infections in all T_tE *B. germanica* groups (Fig. 1).

The effects of host species on *in vitro* excystation of *Gregarina blattarum* are presented in Table 2. Sporozoite excystation was observed in all replications using host gut homogenate extracts of *B. orientalis, P. americana,* and *P. fuliginosa.* Excystation was observed in 83 and 66% of replications using host gut homogenate extracts of *B. germanica* (Fig. 2) and *S. longipalpa,* respectively. No excystation was observed in a neutral buffered saline control assay.

DISCUSSION

The failure of *G. blattarum* to establish infections in *S. longipalpa, B. orientalis, P. americana,* and *P. fuliginosa* may be a strain artifact. Host strain susceptibility to infection by an apicomplexan parasite has been demonstrated. Mayberry *et al.* (1982) examined the role of genetic strain in abnormal host susceptibility to eimerian infection and concluded that genetic factors could directly affect host susceptibility. We have tried to minimize the impact of strain induced susceptibility in this study by using free-breeding cockroach colonies established with wild-type strains col-

TABLE 1

Mean Number of Parasites/Cockroach for Experimental Infections of Four Species of Domiciliary Cockroaches with a *Gregarina blattarum* Strain Isolated from *Blattella germanica*[a]

Host species	*n*	T_0 Control	T_t Control	T_t
Supella longipalpa	30	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)
B. germanica Control	30	0.0 (±0.0)	0.0 (±0.0)	24.5 (±18.2)
Blatta orientalis	30	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)
B. germanica Control	30	0.0 (±0.0)	0.0 (±0.0)	26.6 (±18.4)
Periplaneta americana	30	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)
B. germanica Control	30	0.0 (±0.0)	0.0 (±0.0)	19.1 (±12.5)
Periplaneta fuliginosa	30	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)
B. germanica Control	30	0.0 (±0.0)	0.0 (±0.0)	17.9 (±17.9)

[a] Means are followed parenthetically by standard deviations.

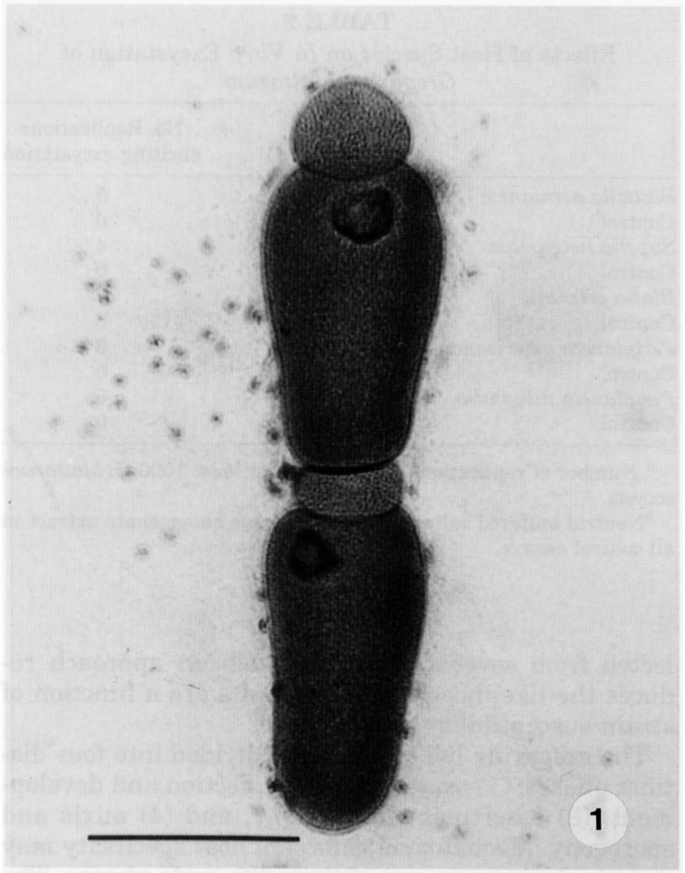

FIG. 1. *Gregarina blattarum.* Gamonts in association, scale bar, 100 μm.

FIG. 2. *Gregarina blattarum. In vitro* excystation in extracts of homogenated *Blattella germanica* alimentary tract; scale bar, 20 μm. Oc, oocyst; Sp, activated sporozoite.

TABLE 2
Effects of Host Species on *In Vitro* Excystation of *Gregarina blattarum*

	k^a	No. Replications eliciting excystation
Blattella germanica	6	5
Control[b]	6	0
Supella longipalpa	6	4
Control	6	0
Blatta orientals	6	6
Control	6	0
Periplaneta americana	6	6
Control	6	0
Periplaneta fuliginosa	6	6
Control	6	0

[a] Number of replications, each testing at least 1000 G. *blattarum oocysts.*
[b]Neutral buffered saline replaced host gut homogenate extract in all control assays.

lected from several localities. Such an approach reduces the likelihood that our results are a function of strain susceptibility or resistance.

The gregarine life cycle can be divided into four distinct phases: (1) excystation, (2) infection and development, (3) assortment and syzygy, and (4) mixis and sporogony. Mechanisms enforcing host specificity may operate during any one of these life cycle phases. The life cycle is intitiated during the excystation phase. Oocysts in the environment are accidentally consumed by a suitable host animal. The enclosed sporozoites activate in the host gut, escape the oocyst sheath, and migrate to the intestinal epithelium. During the infection and development phase, sporozoites establish between lamellar microvilli of the intestinal epithelium (Desportes, 1969; Tronchin and Schrével, 1977). During this growth phase, gregarine trophozoites expand into the intestinal lumen but remain attached to the host epithelium by a mucron or epimerite (Desportes, 1969; Grassé, 1953; Tronchin and Schrével, 1977).

During the assortment and syzygy phase, mature trophozoites form reproductive associations and undergo syzygy to produce a reproductive gametocyst that is shed to the environment with host feces. Gamonts are haploid and eventually undergo gametogony; thus functional gene flow among population members is limited to the assortment and syzygy phase.

The mixis and sporogony phase occurs in the exogenous gametocyst. Gametogony and fertilization give rise to diploid zygotes, each of which forms a protective oocyst envelope and undergoes sporogony to form eight haploid sporozoites. Gametocysts dehisce when mature, releasing infective oocysts into the environment to continue the cycle.

The gregarine life cycle is direct: there is no intermediate host or transmission vector. Host restrictions would be maintained by any mechanism preventing excystation, infection and development, or assortment and syzygy. It is difficult to conceive of a host restriction mechanism operating during the mixis and sporogony phase because this phase occurs exogenously.

The strain of *G. blattarum* isolated from *B. germanica* laboratory colonies at Texas A&M University (TAMU) excysted in host gut homogenate extracts of all cockroach species tested. Thus, host specificity in this system is not the result of restriction mechanisms that prevent excystation. However, the TAMU strain of *G. blattarum* could not establish infections in any of the experimental cockroach species. Mechanisms exist that apparently restrict the infection and development of this *G. blattarum* strain to *B. germanica.* Such a restriction is not consistent with the broad host range previously reported for *G. blattarum* and indicates that *G. blattarum* may comprise a complex of cryptic species.

Levine (1988) concluded that unique host associations were insufficient indicators of species emergence without empirical confirmation. In an earlier work, Levine (1979) noted the strengths of the different host, different parasite model in the presence of cross-transmission studies. Simply, the different host, different parasite model recognizes a failure in parasite transmission and a complete disruption of the parasite's life cycle. Such a failure is of particular evolutionary importance among Eugregarinida. The endogenous parasitic forms (sporozoites, trophozoites, and gamonts) are all haploid: gregarine association and syzygy is the only opportunity for gene flow and mixis within the group. Thus, two gregarine populations unable to establish infections in a common host species are denied the opportunity for gene flow and are reproductively isolated.

We suggest that the *G. blattarum* complex represents a case of incipient speciation in which host-specificity reflects reproductive isolation and physiological divergence has preceded morphological divergence. Additional cross-infection experiments using *G. blattarum* strains isolated from *B. orientalis, P. americana, P. discoidales, P. pennsylvanica,* and *B. craniifer* will uncover additional cryptic species if *G. blattarum* is actually a species complex rather than the single species reported in the literature.

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