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THE ROLE OF PHYLOGENY AND ECOLOGY IN EXPERIMENTAL HOST SPECIFICITY: INSIGHTS FROM A EUGREGARINE–HOST SYSTEM

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ABSTRACT: The degree to which parasites use hosts is fundamental to host–parasite coevolution studies, yet difficult to assess and interpret in an evolutionary manner. Previous assessments of parasitism in eugregarine–host systems suggest high degrees of host specificity to particular host stages and host species; however, rarely have the evolutionary constraints on host specificity been studied experimentally. A series of experimental infections were conducted to determine the extent of host stadium specificity (larval vs. adult stage) and host specificity among 6 tenebrionid host species and 5 eugregarine parasite species. Eugregarines from all host species infected both the larva and adult stages of the host, and each parasite taxa colonized several host species (*Tribolium* spp. and *Palorus subdepressus*). Parasite infection patterns were not congruent with host phylogeny, suggesting that host phylogeny is not a significant predictor of host–parasite interactions in this system. However, the 2 host stages produced significantly different numbers of parasite propagules, indicating that ecological factors may be important determinants of host specificity in this host–parasite system. While field infections reflect extant natural infection patterns of parasites, experimental infections can demonstrate potential host–parasite interactions, which aids in identifying factors that may be significant in shaping future host–parasite interactions.

All parasites exhibit some degree of specificity to their hosts during their life cycles (Rohde, 2002; Caira et al., 2003). For some parasite taxa, the extent of host specificity is even considered a taxonomic character (Levine, 1979). Despite being such a widely recognized and important aspect of parasitism, little is known about what determines a parasite's host range (Perlman and Jaenike, 2003). The range of host species infected may be due to phylogenetic, ecological, physiological, and immunological factors. In turn, these factors influence current host range, as well as the potential host range of parasites.

A growing body of coevolutionary work has addressed host specificity from a phylogenetic perspective and has emphasized the use of both host and parasite phylogenies (Adamson and Caira, 1994; Caira et al., 2003; Poulin and Mouillot, 2003). This approach is important because certain parasite characteristics, such as host range, may be products of common ancestry, which only phylogenetic methodology can detect (Adamson and Caira, 1994). Once developed, the phylogenetic hypotheses place other potentially important factors affecting host specificity, such as adaptation in response to host ecology, into an evolutionary context that can suggest other potentially important influences on the evolution of host specificity.

Determining the causal mechanisms underlying host specificity will be impossible in the absence of information about host specificity (Brooks, 2003). In most cases, host specificity is inferred from previously published reports and surveys across a wide array of host and parasite taxa (Poulin, 1992; Poulin, 1997; Caira et al., 2003). However, data collected in this manner may not reflect host specificity appropriately (Brooks, 2003; Collins and Janovy, 2003). Alternatively, experimental approaches offer an opportunity to systematically study host specificity in the absence of geographic or ecological constraints.

Characteristics amenable to experimental host specificity research can be evaluated by the eugregarine–tenebrionid beetle model system. Eugregarines are apicomplexan parasites (Phylum Apicomplexa, Class Conoidasida, Order Eugregarinorida) of many invertebrate species and, like most apicomplexans, are often assumed to be very host specific (Perkins et al., 2000). However, with few exceptions, host specificity of most gregarines and other apicomplexans is unknown (Levine, 1988).

The present study defines host specificity as the number of host species a parasite can colonize and recognizes that specificity involves consideration of a parasite's ability to establish, develop, and reproduce inside or on the body of the host. Using 6 tenebrionid host species (*Tribolium* spp. and *Palorus subdepressus*) and 5 eugregarine parasite species (*Gregarina* spp. and *Awrygregarina billmani*), 3 main objectives were addressed: (1) establish the extent of host stadium specificity (same species, but different host life cycle stage) with homologous cross infections among the gregarines and the tenebrionid species; (2) experimentally evaluate the extent of host specificity among these species of gregarines to determine whether they follow presumptions of strict host specificity suggested by the literature; and (3) determine whether phylogenetic constraints play a role in the observed host specificity in the eugregarine–tenebrionid model system.

METHODS AND MATERIALS

Host–parasite system

The confamilial hosts (Tenebrionidae: Coleoptera) included 5 *Tribolium* congeners and 1 *Palorus* species: *Tribolium castaneum* Herbst 1797, *Tribolium madens* Charpentier 1825, *Tribolium brevicornis* Leconte 1859, *Tribolium confusum* Jacquelin Du Val 1868, *Tribolium freemani* Hinton 1948, and *P. subdepressus* Wollaston 1864. The confamilial gregarine species (Gregarinidae) consisted of 4 *Gregarina* congeners and 1 *Awrygregarina* species: *Gregarina minuta* Ishii 1914, *Gregarina confusa* Janovy et al. 2007, *Gregarina cloptoni* Janovy et al. 2007, *Gregarina palori* Janovy et al. 2007, and *Awrygregarina billmani* Janovy et al. 2007.

Flour beetles were acquired from research stocks and maintained in plastic jars (100 ml) at the University of Nebraska–Lincoln (see Janovy et al., 2007 for colony origins). The medium in each jar consisted of a 98% 1:1 mixture of whole wheat flour and wheat bran, 1% bakers' yeast, and 1% commercial wheat germ. The stock colonies were kept in a moist incubator at 28 C.

Experimental design

Uninfected adult hosts were obtained by removing pupae from laboratory colonies. Each pupa was washed with distilled water in a strainer and gently blotted dry to remove any infective oocysts that may have been on the surface of the pupae. Individual pupae were then placed into sterilized glass vials (1 ml) containing 1 g of sterile medium (1:1 whole wheat flour and wheat bran). Vials were loosely capped to allow

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air flow, secured within wooden holders (drilled boards), and stored in covered plastic storage boxes at room temperature. This method decreased the probability of oocyst contamination from the air.

Each experiment consisted of an experimental group (T_{exp}), 2 control groups (T_0 and T_t), and a positive control group. Prior to the start of the experiment, beetles from a time zero control group (T_0) were dissected to ensure individuals were uninfected. A second group of individuals from the same source as the T_0 control were placed in new sterilized vials with an uncontaminated piece of apple or potato. This group was then separated into 3 subgroups, i.e., a T_t control group that was maintained throughout the experiment to detect any accidental infection, a positive control group (homologous infection) that was exposed to the parasite to ensure oocyst viability, and an experimental group (heterologous infection). Both the homologous and heterologous infections were obtained using 2 methods.

Infection methods

Infection method 1 (Using sporulated gametocysts): Gametocysts were collected from host larvae by isolating 20 individuals in the moat of a plastic center well 60 × 15 mm Falcon style 3010 organ tissue culture dish, hereafter called a well dish. Each well dish contained a crushed bran flake in the moat and a small section of wet paper towel in the center well. After 24 hr, well dishes were examined for presence of gametocysts within the feces from the larvae. With a moist single-hair paintbrush, gametocysts were removed from the frass and placed onto a moistened black construction paper disc (black dot) produced by a paper punch. One black dot was placed in the center well of each well dish, each outside moat was filled with ~1 ml of water, and the well dishes were covered and set aside for 72 hr, after which the well dishes were observed for sporulated gametocysts. If oocyst chains were present, water was removed from the moat and the well dish was stored at room temperature.

The infective material for an experimental group (T_{exp}) was prepared by gently adding oocysts from 2–4 sporulated gametocysts to a 3 × 3 mm piece of apple or potato. The number of oocysts per gametocyst was unknown for this study, although gametocysts from a related species *Gregarina niphandrodes* contained approximately 3,500 oocysts (Schwank, 2004). Pieces of contaminated potato or apple were placed into a sterilized glass vial (1 ml) with 1 uninfected adult beetle.

Infection method 2 (Direct exposure to infected larvae): Infected larvae were directly removed from a stock culture, rinsed with distilled water to remove external oocysts, and placed in sterilized plastic containers (28 mm × 29 mm) with adults from the experimental and T_t control groups. Control larvae were dissected from these stocks to ensure that the larvae placed in the plastic containers were infected. Similar experimental methodology was followed as in Infection method 1, except that control and experimental groups were maintained in plastic containers of a larger volume as opposed to the smaller glass vials.

For both infection methods, T_t controls and T_{exp} groups were stored in covered plastic storage boxes for 8 days at room temperature and then dissected. The adult head was removed and the intact gut was gently pulled from the body. The gut was placed into *Tenebrio molitor* muscle saline (Belton and Grundfest, 1962), teased apart, and examined using a 10× compound microscope at a total magnification of 100×. If gregarines were observed, the slide was videotaped using a Nikon Alphaphot-2 compound microscope using 10× and 40× objectives, a MicroImage Video Systems YC/NTSC 470-line horizontal resolution camera, and a Panasonic S-VHS recorder.

Infection patterns and host phylogeny

Results from the experimental infections were mapped onto a previously published host phylogeny (Meštrović et al., 2006) to understand the phylogenetic context of host stadium specificity and host specificity for this particular gregarine–tenebrionid model system.

Gametocyst shedding between adult and larval host stages

The colonizing dynamics of gregarines were contrasted amongst adults and larvae by comparing the number of gametocysts shed and mean abundance of parasite infection of larval and adult hosts. Twenty adults were placed into the moat of a well dish, and 20 larvae were placed into the moat of a well dish. Both adult and larval individuals were taken from stock colonies. In each of the well dishes, 1 bran flake was placed in the moat and a wet section of paper towel was placed in the center well. After 24 hr, each well dish was checked for presence of gametocysts in the host feces. The number of gametocysts from each well dish was recorded. After all gametocysts were removed, individuals from each well dish from both adult and larva groups were dissected following the methodology listed above.

Statistical analysis

Data from experimental infections (including both infection methods) were nonnormally distributed; however, homogeneity of variance was similar among groups (Sokal and Rohlf, 1981). Therefore, a Kruskal–Wallis test was performed to evaluate any potential differences between the T_0, T_t, and T_{exp} groups. The Mann–Whitney *U*-test was used to determine whether there were differences among the adult and larval infections. All statistical analyses were executed using Statview 5.0.1 and were deemed significant at $P < 0.05$.

RESULTS

Forty-eight infection assays were performed. Twenty of 22 homologous experimental infections and 22 of 26 heterologous experimental infections were considered valid because the T_0 and T_t controls were uninfected or the mean abundance of infection was extremely low. In addition, heterologous infections were only considered valid if the first condition was met, and a simultaneously run homologous infection (positive control) was infected.

There were no significant differences in parasite mean abundance between the infection methods. For example, the homologous infection *G. palori*:*P. subdepressus* did not differ between the first or second infection method (Mann–Whitney *U*-test: $z = -0.577$, $P = 0.5677$). Comparisons among the 3 groups in both homologous and heterologous infections are summarized in Table I. Of the total homologous experiments performed, statistically significant differences among the 3 groups occurred in at least 1 of the homologous infections for all parasite species ($P < 0.05$). Heterologous infection success varied, with statistically significant differences among the 3 groups occurring in assays where *G. cloptoni* infected *Tr. confusum* and *Tr. castaneum*, and *G. palori* infected *Tr. confusum*. Several heterologous experiments could not be statistically analyzed because mean abundance was equal to zero in all 3 groups. Notably, *Tr. madens* was not naturally infected by a parasite and was also not a suitable host for the parasite species in this study.

Extent of experimental host stadium specificity and host specificity

All homologous experimental infections resulted in gregarine infections of the target hosts (Table II). Therefore, no eugregarines were stadially specific to any host species in this study. Heterologous infections showed that some gregarine species could infect a range of host species (Table II). For example, *Gregarina minuta* infected 2 hosts, i.e., *Tr. confusum* and *Tr. castaneum*, whereas *Awrygregarina billmani* infected 4 hosts, namely, *Tr. confusum*, *Tr. castaneum*, *P. subdepressus*, and *Tr. brevicornis*.

Host phylogeny and resulting infection patterns

The host phylogeny used in this study was that of Meštrović et al. (2006) and was derived from combined segments of CO1 and 16S rDNA. The gregarine species parasitizing the ingroup

TABLE I. Mean abundances of T_0 and T_t controls and T_{exp} group and Kruskal-Wallis corrected for ties, H values and P values are reported. Hm = homologous infection and Ht = heterologous infection.

Parasite	Host	Hm/Ht	Mean abundance			Kruskal-Wallis	
			T_0	T_t	T_{exp}	H	P
G. minuta and *G. confusa*	*Tr. confusum*	Hm	0.67	0.14	3	6.203	0.0450*
G. minuta and *G. confusa*	*Tr. brevicornis*	Ht	0.625	0.125	0	0.65	0.7226
G. minuta	*Tr. castaneum*	Hm	0	0	64	14.269	0.0008*
		Hm	1.7	0	16.4	7.335	0.1969
G. minuta	*Tr. brevicornis*	Ht	0.9	6.6	0	7.95	0.159
G. minuta	*Tr. madens*	Ht	0	0	0	—	—
A. billmani	*Tr. brevicornis*	Hm	0	0.5	44.3	10.629	0.0049*
		Hm	0	7.3	18	6.716	0.0348*
		Hm	0	0	1.9	4.138	0.1263
		Hm	0	0	0.316	1.0	0.6065
A. billmani	*Tr. confusum*	Ht	0	0	1.6	3.733	0.1546
A. billmani	*Tr. castaneum*	Ht	0.2	0	0.4	3.936	0.1398
A. billmani	*Tr. freemani*	Ht	0	0	0	—	—
A. billmani	*P. subdepressus*	Ht	0	0	3.68	5.847	0.0537
G. cloptoni	*Tr. freemani*	Hm	0.3	0	5.8	2.003	0.3664
		Hm	0	0	0.1	2.857	0.2397
		Hm	0	0	0.4	2	0.3679
		Hm	0.7	0	3.5	6.423	0.0403*
		Hm	0	0	1	4.138	0.1263
		Hm	0	0	2.83	6.714	0.0348*
		Hm	0	0	10.2	2	0.3679
		Hm	0	0	13.47	1.985	0.7385
G. cloptoni	*Tr. confusum*	Ht	6.1	0	13.4	8.044	0.0179*
G. cloptoni	*Tr. castaneum*	Ht	0.7	0	0	—	—
		Ht	0	0	60.74	15.161	0.0005*
G. cloptoni	*Tr. castaneum*	Ht	0	0	44.89	19.314	<0.0001*
G. cloptoni	*Tr. brevicornis*	Ht	0	2.8	0	4.138	0.1263
		Ht	0	0	0	—	—
G. cloptoni	*Tr. madens*	Ht	0	0	0	—	—
		Ht	0	0	0	—	—
G. palori	*P. subdepressus*	Hm	0	0	0.67	2.222	0.3292
		Hm	0	0	2	6.197	0.0451*
		Hm	0.4	0	0.8	1.29	0.7316
		Hm	0	0	0.3	2	0.3679
		Hm	0	0	1.47	4.8	0.3084
G. palori	*Tr. confusum*	Ht	0	0	6.65	6.824	0.0330*
		Ht	0	0	10.9	6.495	0.0899
		Ht	0	1.2	0	2	0.3679
G. palori	*Tr. brevicornis*	Ht	0	0	0	—	—
G. palori	*Tr. brevicornis*	Ht	0	0	0	—	—
		Ht	0	0	0	—	—
G. palori	*Tr. freemani*	Ht	0	0	0	—	—

* Indicates that the 3 groups are significantly different from each other ($P < 0.05$). A dash indicates that no statistical test was possible because of zeros in the dataset.

(*Tribolium* spp.) were not host stadium specific; in all cases, parasites were from larvae infected adults of the same host species. In addition, we found that parasites could infect phylogenetically distinct host groups (Fig. 1). *Gregarina minuta* could infect *Tr. confusum* and *Tr. castaneum*, hosts that are members of 2 distinct species-groups and 2 separate clades. However, this species could not infect members of the *brevicornis* group (*Tr. brevicornis*) or the remaining member of the *castaneum* group (*Tr. madens*). Further comparisons between host phylogeny and host specificity demonstrated no clear patterns, which clearly indicated that the eugregarine parasites were infective to host species other than their natural ones (sources of parasites) (Fig. 1).

Gametocyst shedding between adult and larval host stages

After 24 hr, adults shed significantly fewer gametocysts than larvae (Mann–Whitney U-test, *Tr. freemani*, $z = -2.702$, $P = 0.0069$; *P. subdepressus*, $z = -3.240$, $P = 0.0012$; *Tr. confusum*, $z = -2.611$, $P = 0.0090$) (Table IIIa). In addition, *Tr. castaneum* adults tended to shed fewer gametocysts than larvae (Mann–Whitney U-test; $z = -1.928$, $P = 0.0539$) during this same time period.

Following the 24-hr shedding period, gregarines from *Tr. freemani*, *P. subdepressus*, and *Tr. confusum* larvae had a higher mean abundance than did their corresponding adults (Mann–

TABLE II. Results of experimental infections. ''Yes'' indicates that the cross resulted in the presence of trophonts and/or gamonts. ''No'' indicates that the cross resulted in no infection of either trophonts and/or gamonts. Parasite species (PS) are reported with the source host species (SH), which were the origin of the oocysts. Dashes (—) signify that experimental infections were not attempted.

		Target host species					
		Tr. confusum	*Tr. castaneum*	*Tr. brevicornis**	*Tr. freeman*	*P. subdepressus*	*Tr. madens*
PS:	*G. minuta* and *G. confusa*	Yes	—	No	—	—	—
SH:	*Tr. confusum*						
PS:	*G. minuta*	—	Yes	No	—	—	No
SH:	*Tr. castaneum*						
PS:	*A. billmani*	Yes	Yes	Yes	No	Yes	—
SH:	*Tr. brevicornis*						
PS:	*G. cloptoni*	Yes	Yes	No	Yes	No	No
SH:	*Tr. freemani*						
PS:	*G. palori*	Yes	—	No	Yes	Yes	—
SH:	*P. subdepressus*						

* Infections with *Tr. madens* were abandoned because their stock colonies were uninfected.

Whitney *U*-test: $z = -2.278$, $P = 0.0227$; $z = -2.694$, $P = 0.0071$; $z = -2.193$, $P = 0.0283$, respectively) (Table IIIb). After the shedding period, *Tr. castaneum* larvae tended to have higher infection levels than adults (Mann–Whitney *U*-test; $z = -1.776$, $P = 0.0758$).

DISCUSSION

The present study uses experimental and phylogenetic methodologies to understand the evolution of specificity in gregarines. This multifaceted approach emphasizes first, a methodical determination of host range among several parasite taxa and, second, the role phylogenetic constraints play in host specificity. For this study, experimentally derived data concerning host range were directly mapped onto a host species cladogram published by Meštrović et al. (2006). This phylogenetic analysis improves upon a previous molecular *Tribolium* phylogeny (Juan et al., 1993) by providing well-supported ''castaneum'' and ''confusum'' clades and resolving several relationships between species. However, the relationship of *Tr. brevicornis* to the other *Tribolium* clades remains unclear and should be resolved with future work.

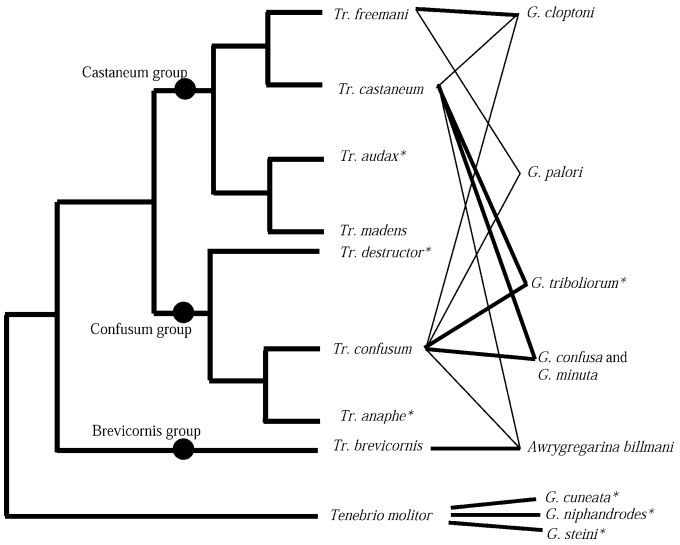

FIGURE 1. Host specificity of gregarines compared with host phylogeny of Meštrović et al. (2006). The black circles denote the 3 host groups that include the ''castaneum'' clade, ''confusum'' clade, and ''brevicornis'' clade, which were previously recognized as distinct groups according to morphological characteristics and geographical distributions (Hinton, 1948). Asterisks indicate host species not included in this study and results for parasites from previous studies (Clopton et al., 1992; Watwood et al., 1997). Thick lines signify the source (natural) host for parasite species.

Two levels of host specificity were examined in this study, including specificity at the host stage and species levels. Clopton et al. (1992) were among the first to address experimentally the extent of stadium specificity in a gregarine–tenebrionid model system. Their study indicated that 3 gregarine species (*Gregarina steini*, *Gregarina cuneata*, and *Gregarina polymorpha*) colonized only larval *Te. molitor*, and 1 gregarine species (*G. niphandrodes*) colonized only the adult stage. Consequently, the strict stadium specificity (not often observed in other host–parasite systems) was initially considered a signature characteristic of the gregarine–tenebrionid model system (Clopton et al., 1992). Recent molecular work by Leander et al. (2003) suggested that morphological characters may be suspect as taxonomic characters within species of *Gregarina*. However, in the study experimentally demonstrating strict host stadium specificity (Clopton et al., 1992), species were distinguished not only on trophont and gamont structure, but also on oocyst shapes and sizes. A second experimental study found neither stadium specificity nor host specificity between 2 hosts, *Tr. confusum* and *Tr. castaneum*, and the parasite *Gregarina triboliorum* (Watwood et al., 1997), thereby demonstrating that stadium specificity was not universal among the gregarines in this tenebrionid–host system.

The current study expands upon the previous studies by establishing the extent to which host stadium specificity occurs among a wider range of related hosts and their parasite taxa. Holometabolic insect hosts that have been used to experimentally determine host stadium specificity include 5 congeneric hosts (*Tribolium* spp.) and 7 confamilial host species (5 *Tribolium* spp., *P. subdepressus*, and *Te. molitor*). Furthermore, this study extends the number of gregarine taxa to 8 *Gregarina* spp. and 1 confamilial parasite, *Awrygregarina*.

Host stadium specificity represents a type of extreme restric-

TABLE IIIa. Mean abundance of gametocysts shed for adults and larvae (n = 20 per well dish for each host stage).

Host species	Mean gametocysts shed by adults	Mean gametocysts shed by larvae	Number of well dishes examined
Tr. freemani	0.08	5.81	11
P. subdepressus	0	4.65	8
Tr. confusum	1.41	38.17	5
Tr. castaneum	0	32.82	5

TABLE IIIb. Numbers of gregarines (trophonts and/or gamonts) after 24-hr shedding period per well dish of adults (n = 20) and per well dish of larvae (n = 20).

Host species	Mean gregarine infection within adults	Mean gregarine infection within larvae	Number of well dishes examined
Tr. freemani	3.85	59.04	11
P. subdepressus	0.28	27.81	5
Tr. confusum	35.96	386.4	5
Tr. castaneum	21.22	407.84	5

tion, or a level of specificity beyond the species level and thus could have important evolutionary implications for gregarines. This is particularly true if each holometabolic insect species represents 2 distinct environments to be potentially colonized. Thus, consideration of host stadium specificity broadens the conceptual basis of host specificity in general. The 5 eugregarine species in this study were not host stadium specific since all the involved gregarine taxa infected both the larval and adult host stages of the host species they normally infect in the lab. Thus, the occurrence of host stadium specificity remains restricted to gregarines from *Te. molitor* as demonstrated experimentally by Clopton et al. (1992). There are no records of host stadium specificity occurring in any other septate gregarine taxa or any other gregarine taxa, including all other members of Subclass Gregarinasina. However, this observation may be misleading, since most gregarine taxa are not well studied beyond their species descriptions, so future reports of host stadium specificity among gregarine taxa are certainly plausible.

The present study also determined the extent of host specificity among host species for several eugregarine species. The approach was unique in that a range of host species and parasite taxa were studied experimentally. The gregarine taxa in this study could be classified as stenoxenous because the eugregarine species were found to infect numerous host species within a single host genus, a situation that is similar to that previously reported for other gregarine taxa (Perkins et al., 2000). At the host genus level, each of the 5 gregarine species infected 2 or 3 *Tribolium* spp., and 2 of the gregarine species also infected a noncongeneric host species (*P. subdepressus*). Therefore, the parasite species were similar in terms of the number of host species that each could colonize, but differed in the individual host species that they colonized. No attempt was made to experimentally infect other host families with the gregarine species, although such experiments would be an interesting test of the extent of ecological host specificity because many tenebrionid species have ranges and niches that overlap with other coleopteran families.

The extent of host specificity in apicomplexans was previously described as mesoxenous (occurring in hosts of a single order) and stenoxenous (Levine, 1985; Clopton et al., 1992; Perkins et al., 2000). However, describing host specificity in these terms lacks evolutionary perspective and, therefore, does not aid in efforts to elucidate the constraints on and avenues for evolutionary change. By comparing the host phylogeny with experimentally determined specificity of parasite taxa, the avenues for, and constraints on, evolutionary change can be postulated. In the present study, it was found that presumably closely related septate gregarine species did not experimentally infect their host species according to phylogenetic relationships. The experimental host specificity data showed that each host congener was colonized by a unique set of parasite species and this pattern did not follow phylogenetic relationships among the hosts. Given that the phylogenetic background of the host was not sufficient to explain the observed host specificity, the data concerning the colonizing dynamics of eugregarines suggest an alternative hypothesis.

In the present study, the difference in gametocyst shedding and level of infection between adult and larval stages is reported for the first time. Larval hosts had significantly different infection levels and different outputs of parasite propagules into the environment. Larvae of *Tr. freemani*, *Tr. confusum*, and *P. subdepressus* not only shed more gametocysts than adults but also were significantly more infected after the 24-hr shedding period. A fourth species, *Tr. castaneum*, exhibited the same trend, but the difference was not statistically significant, most likely due to the high variation levels that often characterize gregarine infection levels. These results suggest that the differential colonizing dynamics of the host stages may have important implications for parasite propagule dispersal (oocysts) and ultimately gregarine host specificity.

The immediate dispersion of oocysts is reliant on the distribution of the beetle stages within the environment. Once airborne, adult *Tr. castaneum* and *Tr. freemani* can fly well (Barnes and Kaloostian, 1940; Graham, 1962; Imura, 1987). In contrast, other species, including *Tr. confusum* and *Tr. brevicornis*, are not known to fly (Good, 1933; Mulder and Sokoloff, 1982). Interestingly, in our study, the adult stages that are more likely to colonize new populations than the larval stages were the least infected and had the slowest rate of parasite propagule (gametocyst) release. This observation suggests that the geographic dispersal of gregarine infections is probably most dependent on external transmission factors such as wind and water, or attachment to the external surface of the adult.

The observation that some gregarine species can mature and develop in a range of host species independent of phylogenetic relationships suggests that if oocysts are encountered and consumed they will mature and develop in numerous host species, and also suggests that when various species are not infected, the reason may be ecological instead of phylogenetic. *Tribolium* species may have similar physiological characteristics or resources for gregarine species because they are closely related host species, but the likelihood of infection may be more dependent upon the ecological interactions of the hosts.

Historically, *Tribolium* species were postulated to occur in geographically isolated species-groups, but now many members

of these species-groups are cosmopolitan in distribution as a result of human agriculture–related activities (Hinton, 1948). When mapped to host phylogeny, the pattern of host specificity determined in the present study may reflect the currently overlapping niches of these once geographically separate species-groups. Although no further investigation of colonizing dynamics beyond gametocyst shedding and mean abundance of infection was conducted in this study, overall, the colonizing dynamics of host species may have important implications for host specificity. An understanding of the mode of dispersal for oocysts in the environment, particularly in nature, may lead to important discoveries that provide more evidence for the constraints on and avenues for gregarine evolution in particular and apicomplexan evolution in general.

The present work contributes to the short list of studies that investigate the extent of host specificity among numerous parasite taxa using experimental and phylogenetic approaches. This scheme ensures that the results are not a product of the methods, as is often the case in studies that are ecological by design. More specifically, an experimental approach allows one to test all possible combinations of host–parasite interaction. By testing host specificity experimentally, the level of specificity resulting from such a study is not necessarily limited by ecological host–parasite interactions, but may ultimately reflect such interactions.

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