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GREGARINES ON A DIET: THE EFFECTS OF HOST STARVATION ON *GREGARINA CONFUSA* JANOVY ET AL., 2007 (APICOMPLEXA: EUGREGARINIDA) IN *TRIBOLIUM DESTRUCTOR* UYTTENBOOGAART, 1933 (COLEOPTERA: TENEBRIONIDAE) LARVAE

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ABSTRACT: This study was designed to explore the nutritional relationship between *Gregarina confusa* (Apicomplexa: Eugregarinida) parasites and its coleopteran host, *Tribolium destructor*, by measuring the cytoplasmic density of gregarines in continuously fed larvae, starved larvae, and larvae refed after starvation. Cultures were maintained in a standard media (whole wheat flour:commercial wheat germ:yeast [30:10:1]). Larvae from control and experimental groups were dissected daily for 3 days then allowed to feed or starve for an additional 3 days. On day 6, the remaining experimental larvae were divided and placed into 2 groups; 1 group remained starved while larvae from the second group were fed a Wheaties® flake. Photographs were taken of the parasites daily and analyzed using ScionImage™. Gregarines from starved larvae were significantly longer and skinnier than those from fed controls, and there was also a significant difference between gregarine deutomerite cytoplasmic densities. Parasites from refed larvae regained cytoplasmic density within 24 hr and showed morphological similarities to those from fed larvae. This study shows that the *Tribolium destructor–Gregarina confusa* relationship can be manipulated easily through alterations of host diet and thus is an excellent model for use in the study of chemical relationships between parasites and their hosts.

Gregarines are apicomplexans that occur in most, if not all, invertebrate groups, but are especially common in arthropods and annelids. Because their hosts are so numerous, gregarines should probably be considered the most diverse group of parasites (Roberts and Janovy, 2005). A typical gregarine life cycle includes trophont and gamont stages, which participate in the processes of association, syzygy, gametogenesis, gametocyst formation, fertilization, oocyst formation, and dehiscence. Oocyst exsporulation, attachment to host intestinal epithelium, penetration of epithelial cells, growth, gamont association, syzygy, and gametocyst formation all occur within the host. Because both hosts and parasites are so diverse, gregarine-invertebrate systems promise to be a rich source of material for exploring the evolution of host-parasite relationships.

There have been numerous cytochemical studies on gregarines (Göhre, 1943; Schrevel, 1970; Amoji, 1975; Ramachandran, 1976; Sathananthan, 1977; Janardanan and Ramachandran, 1987), but none has focused on the effects of host diet on parasite morphology or cytoplasmic contents. Studies by Schawang and Janovy (2001) showed that starvation of adult *Tenebrio molitor* (Tenebrionidae) results in a halt of gametocyst shedding by *Gregarina niphandrodes*. Thus, by altering diet, the life cycle could be ‘‘turned off’’ at the gametocyst life cycle stage. Once the hosts were refed, gametocyst shedding resumed. These observations suggest that metabolic relationships between host and parasite could be explored with simple techniques. The current study is an attempt to extend the Schawang and Janovy (2001) effort by using a different host-parasite system, focusing on the trophont and gamont life cycle stages, and addressing the buildup of iodine-positive materials, presumably carbohydrate, in parasite cytoplasm. This investigation is thus the first step toward using such systems to explore host-parasite interactions at the molecular level.

The following questions were addressed: (1) Does host starvation affect buildup of iodine-positive materials in the parasite cytoplasm? (2) Does host starvation affect parasite morphology? And (3) if there are observed effects of host starvation on parasite cytoplasm or morphology, can those effects be reversed by refeeding the host?

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MATERIALS AND METHODS

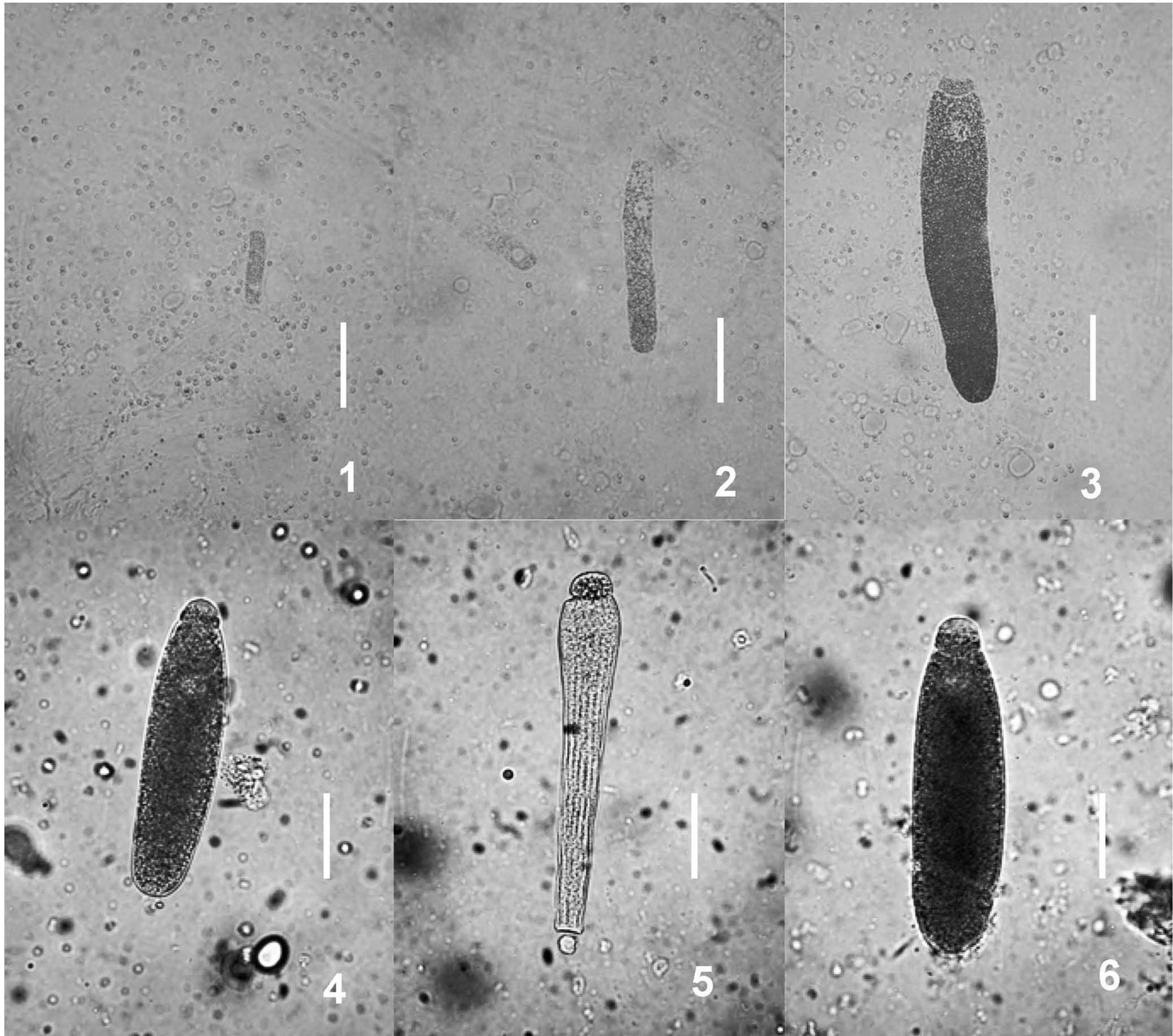
Cultures of *Tribolium destructor* Uyttenboogaart, 1933, were obtained from Andrea Valigurova, Department of Zoology and Ecology, at Masaryk University in the Czech Republic on 8 July 2003, and subsequently maintained in plastic jars 5 cm in diameter and 5 cm tall with perforated plastic lids in an incubator at 27 C with a pan of water provided for moisture. Culture medium consisted of wheat bran, whole wheat flour, commercial wheat germ, and baker's yeast (40:40:15:5). New cultures were started by transfer of 20 larvae and 20 adults every 2 wk. Under these conditions, offspring larvae became very heavily infected within a month. Host voucher specimens were killed by freezing then glued to pin points (adults) or killed by immersion in boiling water then preserved in 70% ethanol (larvae).

Gregarines were identified as *Gregarina confusa* Janovy et al., 2007, based on size, body proportions, epimerite shape, and oocyst size. For voucher specimens, larval hosts were dissected and stained smears were made from guts that had been teased apart by the methods of Kula and Clopton (1999). Air-dried smears were fixed in alcohol-formalin-acetic acid, washed in 70% ethyl alcohol, stained in Semichon's acetocarmine, dehydrated, cleared in xylene, and mounted with Damar balsam (Spectrum, Gardena, California) (Pritchard and Kruse, 1982; Kula and Clopton, 1999). Such smears were also made using guts from larvae dissected in 1:3:Mayer's albumin:water, stained with Lugol's iodine solution, then dehydrated and mounted as mentioned above. Fresh preparations were made in *Tenebrio* sp. saline (Belton and Grundfest, 1962).

For measurements of living organisms, images were taken with a Nikon Coolpix 995 camera fitted with an Optem 25-70-14 adapter (www.optemintl.com). Digital photograph numbers were recorded, and the actual lengths of specimens were calculated from photographs of a stage micrometer. These photographs were then converted to bitmaps, 15.2 cm × 11.4 cm at 39.4 pixels/cm, and adjusted to auto levels using Adobe Photoshop 7.1™ (Adobe Systems Inc., San Jose, California). ScionImage™ (Scion Corporation, Frederick, Maryland) image analysis software was then used on these pictures to measure cytoplasmic densities, lengths, and widths of both the protomerite and deutomerite of trophonts and gamonts. Photographs of fixed and stained specimens were taken by the same camera; Adobe Photoshop 7.1™ and Adobe Illustrator 10.0.3™ were used to prepare the figures.

EXPERIMENTAL DESIGN

Larvae were taken from cultures approximately 1-mo-old and dissected to verify heavy gregarine infections (>500/individual). A single trial involved 3 groups of at least 5, 9–10 mm



FIGURES 1–6. Gregarines from *Tribolium destructor* larvae. (**1–3**) *Gregarina confusa* of various ages stained with Lugol's iodine solution, showing increasing accumulations and concentration of polysaccharide with increasing size. (**4**) Live *G. confusa* from time 0 fed control *Tr. destructor* larva. (**5**) Live *G. confusa* from *Tr. destructor* larva starved for 7 days. (**6**) Live *G. confusa* from *Tr. destructor* larva starved for 6 days then refed Wheaties® flake. Bar = 50 μm in all figures.

long, larvae. Included were a time-zero (T_0) control group, a starved experimental group, and a continuously fed time-end (T_T) control group. The T_T group was fed whole wheat flour, commercial wheat germ, and baker's yeast (30:10:1). All larvae were kept in covered 15 × 60 mm glass Petri dishes on a laboratory shelf for the experiment duration. Each day for 3 consecutive days, 5 larvae were dissected from both the starved and T_T groups using the same protocol as for T_0 larvae. Following the daily dissection, all remaining starved larvae were transferred to clean Petri dishes to minimize the possibility of reinfection. Larvae remained in these dishes for 3 more days; then half the starved group was refed a single Wheaties® flake in a separate Petri dish. On the 7th day, remaining larvae from control fed, experimental starved, and experimental refed groups were dissected using the above protocol. For each group's dissected larvae, at least 20 pictures were taken of gamonts, and 20 pictures were taken of trophonts, resulting in ~100–200 pictures daily.

These experiments were repeated 3 times. Measurements of lengths, widths, and densities were obtained using image analysis software and copied into Excel™ spreadsheets (Microsoft Inc., Redmond, Washington); statistical analysis was performed using the built-in Excel™ functions.

Parasite voucher specimens (6 slides stained with acetocarmine, 1 slide stained with Lugol's iodine) were deposited in the Harold W. Manter Laboratory of Parasitology collection, University of Nebraska–Lincoln, with accession numbers HWML48550 and HWML48550, respectively. Voucher sym-

TABLE I. Comparisons of the deutomerites' mean cytoplasmic density (number of parasites measured, standard deviation) in ScionImage™ units between gregarines from control fed versus experimental starved larvae, with day 7 control data taken of gregarines from the refed experimental larvae.

Trial	Primite		Satellite		Trophont	
	Day 3	Day 7 refed	Day 3	Day 7 refed	Day 3	Day 7 refed
1 Control-fed	146.9 (21, 17.7)	143.1 (18, 20.2)	155.0 (21, 17.5)	136.0 (18, 30.1)	147.0 (10, 9.9)	142.7 (18, 19.3)
Expt-starved	95.1 (22, 22.1)	160.7 (33, 25.0)	122.4 (22, 22.8)	159.9 (33, 21.8)	98.8 (22, 23.9)	176.0 (20, 30.9)
p/=/*	<0.0001	0.0005	<0.0001	0.0008	<0.0001	<0.0001
2 Control-fed	157.3 (14, 17.1)	151.7 (38, 33.1)	164.7 (14, 18.6)	157.2 (38, 19.3)	163.4 (10, 23.7)	163.9 (32, 19.7)
Expt-starved	102.4 (26, 21.7)	95.4 (13, 15.9)	124.7 (26, 29.6)	117.5 (13, 31.4)	118.9 (44, 26.0)	91.3 (26, 22.2)
p/=/*	<0.0001	<0.0001	<0.0001	0.0003	<0.0001	<0.0001
3 Control-fed	137.2 (42, 32.6)	169.8 (20, 23.2)	133.5 (42, 38.0)	163.1 (20, 23.6)	139.2 (85, 24.7)	177.0 (34, 16.0)
Expt-starved	109.4 (27, 15.9)	102.4 (14, 16.8)	123.9 (27, 21.7)	126.3 (14, 17.5)	113.3 (44, 20.7)	113.8 (26, 23.1)
p/=/*	<0.0001	<0.0001	0.09	<0.0001	<0.0001	<0.0001

* Probability of equality between gregarines from control fed versus starved larvae as determined by *t*-test; measurements of parasites from all larvae in a group are pooled.

biotype specimens, 5 adults and 10 larvae, were deposited in the Division of Entomology collection, University of Nebraska State Museum, with accession numbers J. Janovy 55-59 for the adults and J. Janovy 60 for the larvae.

RESULTS

Gregarines stained in Lugol's iodine exhibited very dark granulation throughout the deutomerite cytoplasm, although protomerite cytoplasm tended to remain relatively clear (Figs. 1–3). Staining was most dense in larger individuals, and the smallest ones, especially singlet trophozoites, typically had relatively clear cytoplasm. Larger gregarines from continuously fed larvae showed dense cytoplasm, with granules often obscuring the nucleus when observed alive (Fig. 4). In starved larvae, gregarine cytoplasm was generally clearer than in parasites from fed larvae (Fig. 5); refeeding of starved larvae produced gregarines with very high cytoplasmic density (Fig. 6). Larvae isolated without food shed many gametocysts the first day, fewer the second day, and by the third day gametocyst shedding stopped. These results were not quantified, but the cessation of gametocyst shedding by starved larvae was obvious.

Table I shows the cytoplasmic densities and *P*-values from *t*-tests performed between gregarines from control fed and experimental starved larvae. There was a significant difference between cytoplasmic density for gregarines in starved versus continuously fed larvae. This result was consistent for primites and satellites of paired gamonts as well as for trophonts. Although gregarines from control fed larvae retained a high cytoplasmic density (between 110 and 165 SCN units) throughout an experiment, gregarines from starved larvae exhibited a drastic decrease in cytoplasmic density (between 87 and 124 SCN units) by day 3 of the experiments. After refeeding, gregarines from previously starved larvae showed a significant increase in cytoplasmic density in less than 24 hr.

Table II shows parasite length/width ratios and *P*-values from *t*-tests performed between gregarines from control fed and experimental starved larvae. There was a significant difference between the means of these ratios for gregarines in starved versus continuously fed larvae; the difference was due primarily to the loss of width in parasites from starved hosts. This result was true for primites and satellites of paired gamonts as well as for trophonts. After refeeding, gregarines from previously starved larvae showed a significant reduction in length/width ratio, or relative increase in width.

TABLE II. Comparisons of the deutomerites' mean length/width ratios (number of parasites measured, standard deviation) between gregarines from control-fed versus experimental-starved larvae, with day 7 control data taken of gregarines from the refed experimental larvae.

Trial	Primite		Satellite		Trophont	
	Day 3	Day 7 refed	Day 3	Day 7 refed	Day 3	Day 7 refed
1 Control-fed	4.9 (21, 0.4)	4.2 (33, 0.7)	4.9 (21, 0.7)	3.8 (33, 0.8)	5.6 (10, 0.8)	3.9 (20, 0.8)
Expt-starved	6.5 (22, 0.9)	7.3 (8, 0.9)	7.0 (22, 1.0)	6.7 (8, 0.6)	6.2 (23, 1.4)	7.0 (6, 2.1)
p/=/*	<0.0001	0.0005	<0.0001	0.0008	0.05	<0.0001
2 Control-fed	3.6 (14, 0.5)	4.2 (38, 0.7)	3.3 (14, 0.5)	3.7 (38, 0.5)	3.6 (10, 0.4)	3.8 (32, 0.7)
Expt-starved	6.5 (26, 0.9)	6.1 (13, 1.2)	6.2 (26, 1.1)	5.8 (13, 1.1)	5.6 (44, 1.3)	5.1 (26, 0.6)
p/=/*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
3 Control-fed	5.0 (42, 0.8)	4.2 (20, 0.8)	4.9 (42, 1.2)	3.8 (20, 0.9)	5.3 (85, 1.2)	4.0 (34, 0.9)
Expt-starved	6.1 (27, 0.9)	6.0 (14, 1.6)	6.9 (27, 1.4)	6.1 (14, 2.0)	5.7 (44, 1.3)	6.4 (26, 1.4)
p/=/*	<0.0001	0.0005	<0.0001	0.0004	0.01	<0.0001

* Probability of equality between gregarines from control fed versus starved larvae as determined by *t*-test; measurements of parasites from all larvae in a group are pooled.

DISCUSSION

The major contribution of the present study is the demonstration that a protistan parasite's metabolic processes, namely, the accumulation of stored energy reserves, can be halted through manipulation of host diet and can be started, very quickly, by feeding of the host. The host/parasite metabolic interaction is evident through both the change in cytoplasmic density of gregarines due to carbohydrate accumulation, as well as from the morphological changes that occur. Moreover, this metabolic relationship can be easily controlled and manipulated through starvation of a few days duration. These experiments can be performed quickly, at minimal cost, and effectively. This particular host-parasite interaction (*Tr. destructor–G. confusa*) has not been studied before in the metabolic sense, and the results of these experiments pave the way for determining how gregarines rely on their hosts, what happens to gregarines during host starvation, and what diet is optimal for gregarines to thrive.

A major finding from this work is that both the trophont and gamont stages can be manipulated through host starvation. Thus, in addition to the work of Schawang and Janovy (2001), this study shows that all the life cycle stages occurring within the host can be easily manipulated by withholding food from the host. Not only can these life cycle stages be manipulated, but they also can be altered quickly (in less than 24 hr) and predictably. The data also show that gregarines rely on host diet for nutrition and respond to carbohydrates enzymatically degraded by the host by accumulating carbohydrates of their own. The results from this experiment, as well as those from Schawang and Janovy (2001), suggest that the role of carbohydrates may be linked with locomotion or life cycle stage necessary for reproduction, such as gametocyst wall formation, which is essential for gametogenesis.

In addition to a decrease in cytoplasmic density with host starvation, there is also a significant change in morphology. Length/width ratios are routinely used as taxonomic characters in gregarines, but this study shows that such ratios are subject to considerable modification by host dietary experience. One prediction derived from this investigation is that host and parasite dietary requirements are potentially different, at least in some systems; thus, a host might well survive under conditions that modify a parasite's development and morphology, or vice versa. It is also important that the long, skinny gregarines from starved larvae become short, robust, and dense within 24 hr of introducing a Wheaties® flake to the host. This rapid response indicates that assimilative metabolism of the parasite can be studied simply by providing the host with certain known dietary components. Although there are still many questions to be answered regarding the observations reported here, the wealth of gregarine species and extreme diversity of their hosts, combined with a novel approach to the study of host-parasite interactions, means that a rich supply of excellent projects awaits future researchers interested in gregarines.

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LITERATURE CITED

AMOJI, S. D. 1975. On the nature of polysaccharide reserve in the gregarine *Stylocephalus mesomorphi*: A cytochemical study. Acta Protozoologica **13:** 401–408.

BELTON, P., AND H. GRUNDFEST. 1962. Potassium activation and K spikes in muscle fibers of the mealworm larva (*Tenebrio molitor*). American Journal of Physiology **203:** 588–594.

GÖHRE, E. 1943. Untersuchungen über den plasmatischen Feinbau der Gregarinen mit besonderer Berücksichtigung der sexualitätsverhälnisse. Archiv für Protistenkunde **96:** 296–324.

JANARDANAN, K. P., AND P. RAMACHANDRAN. 1987. Observations on the polysaccharides of *Stenoductus carlogoni* Cephalina Monoductidae: A cyto-biochemical analysis. Archiv für Protistenkunde **133:** 145–150.

KULA, R. R., AND R. E. CLOPTON. 1999. *Amoebogregarina nigra* n. gen., n. comb. (Apicomplexa: Gregarinidae from adult *Melanoplus differentialis* (Orthoptera: Acrididae) in southeastern Nebraska. Journal of Parasitology **85:** 321–325.

PRITCHARD, M. H., AND G. O. W. KRUSE. 1982. The collection and preservation of animal parasites. University of Nebraska Press, Lincoln, Nebraska, 141 p.

RAMACHANDRAN, P. 1976. Cytochemical observations on *Stenoductus penneri* (Cephalina: Monoductidae). Zoologischer Anzeiger **197:** 234–237.

ROBERTS, L. S., AND J. JANOVY, JR. 2005. Foundations of parasitology, 7th ed. McGraw Hill, Dubuque, Iowa, 702 p.

SATHANANTHAN, A. H. 1977. Cytochemical nature and fine structure of the gregarine *Zeylanocystis burti* Dissanaike, with special reference to microfilaments, microtubules, and movement. Journal of Protozoology **24:** 233–243.

SCHAWANG, J. E., AND J. JANOVY, JR. 2001. The response of *Gregarina niphandrodes* (Apicomplexa: Eugregarinida: Septatina) to host starvation in *Tenebrio molitor* (Coleoptera: Tenebrionidae) adults. Journal of Parasitology **87:** 600–605.

SCHRÉVEL, J. 1970. Recherches ultrastructurales et cytochimiques sur le paraglycogéne, réserve glucidique des grégarines et coccidies. Journal de Microscopie **9:** 593–610.