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Short Communication

Eugregarines reduce susceptibility of the hide beetle, *Dermestes maculatus*, to apicomplexan pathogens and retard larval development

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ABSTRACT

Eugregarines are abundant in a great diversity of invertebrates, and yet their relationships with their hosts are subject to controversy and confusion. We tested the effect of the eugregarine, *Pyxinia crystalligera*, on growth, development, and susceptibility to two Apicomplexa pathogens of the hide beetle, *D. maculatus*. Heavy infection with eugregarines provided partial protection from two pathogenic members of Apicomplexa, *M. trogodermae* and *A. tribolii*. Infection with *P. crystalligera* caused lower weight in beetle larvae, but did not significantly retard pupation or adult emergence. *A. tribolii* infection of Lepidoptera and *M. trogodermae* infection of *D. maculatus* are reported for the first time.

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1. Introduction

Eugregarines are nearly ubiquitous among insects, and yet the nature of the symbiotic relationship between them and their hosts has been subject to conflicting reports and opinions. They are often referred to as parasites or pathogens, but it is not clear that they have a great negative impact on their hosts (Lange and Lord, 2011). Among eugregarines, deleterious effects on hosts have been demonstrated for aseptate species such as *Ascogregarina* spp. and *Psychodiella* spp. that infect Diptera (Er and Gokce, 2005; Lantova et al. 2011). Based on ribosomal DNA sequences, the pathogenic aseptate species are closely aligned with the neogregarines (Lantova et al., 2010; Votýpka et al., 2009), which are well known pathogens. Aside from the aseptate species, classification of eugregarines as pathogens or even parasites of insects has little empirical support. Indeed, they are frequently referred to as commensals (Lange and Lord, 2011).

We have noted apparently reduced susceptibility to challenge with *Mattesia* spp. of some Coleoptera with heavy eugregarine loads. The purpose of this study was to confirm that eugregarines confer protection against pathogenic Apicomplexa and to assess the effect of eugregarines on growth and development of the hide beetle, *Dermestes maculatus*.

2. Materials and methods

2.1. Organisms

D. maculatus larvae were from a culture that was established in 2009 from an infestation at a western Missouri dog food plant. They were maintained on a diet of ground Purina rice and lamb dog food (Purina, Gray Summit, MO) with rolled oats. Larvae weighing from 5 to 10 mg and 14–18 days of age were used in assays. *Pyxinia crystalligera* (Eugregarinorida), a septate eugregarine, was present with high prevalence in the hide beetles from the time of collection. *Mattesia trogodermae* (Neogregarinorida) was collected from a colony of *Trogoderma variabile* and cultured in that species. *Adelina tribolii* (Eucoccidiorida) was provided by Dr. Tove Steenberg, University of Aarhus, who discovered it in *Ephestia kuhniella* that were fed on California raisins that may have been an infection source. Its identity was determined by morphology, infectivity for *Tribolium castaneum*, and inability to infect *Tenebrio molitor*. It was propagated in *E. kuhniella* and *Plodia interpunctella*. The *P. crystalligera* burden in *D. maculatus* larvae was reduced by submersion of pupae in pH 2.5 10 mM glycine buffer for 7 h, a modification of the procedure of Stanley (1964) or by washing eggs (Kozloff, 1953) and placing in heat-disinfected diet in groups of 50–100.

2.2. Pathogen co-infection assays

Beetle larvae were placed individually in 2 cm^3 wells of polystyrene assay trays (C-D International, Pitman, NJ) with 100–120 mg

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of diet rice and lamb dog food with or without (controls) 2×10^6 oocysts of *A. tribolii* or *M. trogodermae*/g. Prior to each assay, the midguts and hindguts were dissected from 10 larvae each of cultures lightly and heavily infected with *P. crystalligera* to confirm that the lightly infected larvae had fewer than 50 trophozoites and the heavily infected larvae had more than 200 trophozoites. Assays were incubated for three weeks at 30 °C and 50–75% RH. All larvae were then smeared on slides and examined with phase contrast optics at 100 and 400× for the presence of pathogens. At three weeks, most *M. trogodermae* were oocysts, and most *A. tribolii* were in the gamont stage and were located in fat body and other tissues. Each assay was carried out three times (replicates) with 32 larvae/replicate.

2.3. Development, weight, and longevity

Eggs were obtained on faux fur from 36 h oviposition of females from acid-treated pupae. They were washed and placed individually in 1 oz. plastic cups with 1 g of dog food and oats diet with or without 10 mg of frass from larvae and adults of heavily Pyxinia-infected cultures. The larvae were weighed at 15 d from oviposition, and the days to pupation and adult emergence were recorded. Six or more days after emergence, all adults were smeared and examined for the presence of *P. crystalligera*. Any larvae that were reared on frass-free diet that were infected with *P. crystalligera* were excluded from the analysis ($n = 36$ frass-fed infected and 40 frass-free uninfected larvae).

2.4. Data analysis

Control insects in the test of susceptibility to Apicomplexa were not infected by the pathogens, thus they were not included in the analyses. Student's *t* test was used to compare means of normally distributed data. The Mann–Whitney test was used for eugregarine effect on pupation and emergence data, which were not normally distributed with or without transformations.

3. Results

3.1. Pathogen infection

Treatment with *M. trogodermae* resulted in a mean percentage infection of hide beetle larvae with low *P. crystalligera* loads that was 5.6-fold and significantly greater than the mean infection for those with high *P. crystalligera* loads ($t = 4.2$, $df = 4$, $P = 0.013$). Similarly treatment with *A. tribolii* resulted in a mean percentage infection that was 5.4-fold and significantly greater than the mean infection for those with high *P. crystalligera* loads ($t = 9.5$, $df = 4$, $P < 0.01$). (Fig. 1). There was no *M. trogodermae* or *A. tribolii* infection in control larvae, and there was no mortality in any of treatments.

3.2. Development

At 15 days from oviposition, larvae that were heavily infected with *P. crystalligera* weighed significantly less than uninfected larvae ($t = 2.3$, $df = 74$, $P = 0.025$) (Fig. 2). Time to pupation ($T = 1497$, $P = 0.27$) and time to emergence ($T = 1490$, $P = 0.81$) did not differ significantly between heavily and uninfected larvae. The only sex-related significant difference was not symbiont-related. Overall, males completed their development to adults significantly faster than females regardless of the level of eugregarine infection ($t = 3.9$ $df = 74$, $P < 0.01$).

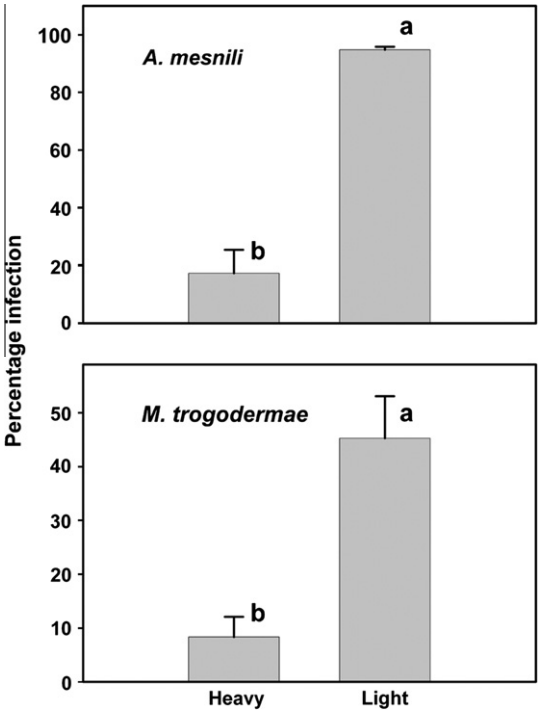

Fig. 1. Prevalence (±SE) of infection with *M. trogodermae* or *A. tribolii* in *D. maculatus* with heavy (>200/larva) or light (<50/larva) *P. crystalligera* infections. Different letters on columns in each graph indicate significant differences in means (Student's *t* test, $\alpha = 0.05$).

4. Discussion

Our results demonstrate that heavy infection with gut-inhabiting eugregarines, *Pyxinia crystalligera*, can have both positive and negative effects on hosts. We found that infected larvae weighed significantly less than those left uninfected. More importantly, heavy infection conferred protection from orally transmitted pathogens, *M. trogodermae* and *A. tribolii*.

Most previous reports on the effects of eugregarines on their hosts investigate impact of natural levels of infection. These studies have not been consistent but generally indicate weakly negative impact of eugregarines and often are associated with suboptimal diet or other stress factors. Sumner (1936) reported that *Tenebrio molitor* growth rates and longevity were greater with *Gregarina steini* than without infection. In contrast, Harry (1967) found no significant effect of *Gregarina polymorpha* on *T. molitor* under optimal rearing conditions, but when larvae were reared under dietary stress, the gregarines had a considerable adverse effect on the final pupal weight and the ability of the larvae to complete development. Some gregarines have negative impacts on their hosts when under dietary stress, resulting in reduced weight and, in at least one case, reduced ability to complete development (Harry, 1967; Dunkel and Boush, 1969; Schwalbe and Baker, 1978). Bouwma et al. (2005) interestingly note that although eugregarine infection negatively correlated with foraging rate and reduced colony fecundity, eugregarine infection positively correlated with adult longevity. They hypothesize that the positive correlation with adult longevity may be due to the fact that infected adults forage less and thus reduce foraging risk. Rodriguez et al. (2007) studied deliberately infected *T. molitor* with adult-specific gregarine, *Gregarina niphandrodes* and found that infection did not significantly affect the population dynamics of *T. molitor*, but heavy infections reduced longevity.

In contrast to the effects of eugregarines on growth and development, interference with apicomplexan pathogens has not been

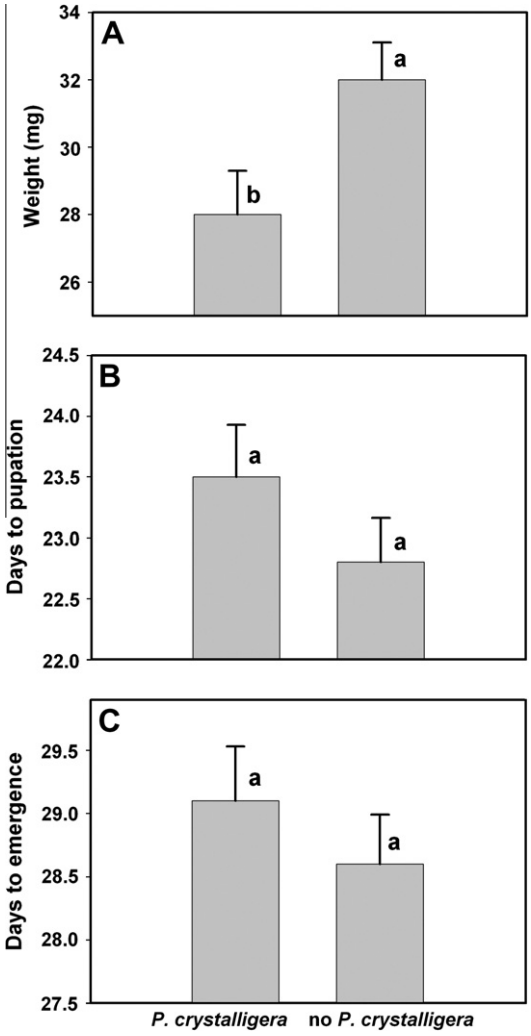

Fig. 2. (A) Weight of larvae 15 days after oviposition, (B) days to pupation, and (C) days to emergence of *Pyxinia crystalligera*-infected and uninfected *Dermestes maculatus*. Columns in each graph with the same letter are not significantly different (Student's t test for weight, Mann–Whitney test for pupation and emergence, $\alpha = 0.05$).

previously reported. The considerable effect of eugregarines on Apicomplexa infection may be of greater importance than the relatively minor effects on growth and development. Perhaps eugregarine infection is an important epidemiological factor in natural insect populations and even an impediment to the efficacy of per os transmitted microbial insecticides.

Both the effect of *P. crystalligera* on larvae size and interference with apicomplexan pathogen may be due to similar mechanisms. One possibility suggested by Harry (1970) is that obstruction of the intestinal tract or gut epithelium. This obstruction can potentially reduce nutrient uptake thereby reducing larval size. Eugregarines may also obstruct the pathogen and thus reduce establishment of the pathogen. Alternatively competition by eugregarine for nutrients and space may reduce larval growth. Similarly competition may reduce pathogen establishment and growth. These two possibilities are not mutually exclusive, and both factors may be involved in the effect of eugregarines on host growth and interference with pathogens.

Previous to this report, *A. tribolii* was only known to infect Coleoptera. Its infectivity for *E. kuhniella* and *P. interpunctella* presents questions about the taxonomic boundaries of *Adelina* spp.

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References

Bouwma, A.J., Howard, K.J., Jeanne, R.L., 2005. Parasitism in a social wasp: effect of gregarines on foraging behaviour, colony productivity, and adult mortality. Behav. Ecol. Sociobiol. 59, 222–233.

Dunkel, F.V., Boush, G.M., 1969. Effect of starvation on the black carpet beetle, *Attagenus megatoma*, infected with the eugregarine *Pyxinia frenzeli*. J. Invertebr. Pathol. 14, 49–52.

Er, M.K., Gokce, A., 2005. Effect of *Diplocystis tipulae* sherlock (Eugregarinida: Apicomplexa), a coelomic gregarine pathogen of tipulids, on the larval size of *Tipula paludosa* meigen (Tipulidae: Diptera). J. Invertebr. Pathol. 89, 112–115.

Harry, O.G., 1967. The effect of a eugregarine *Gregarina polymorpha* (Hammerschmidt) on the mealworm larva of *Tenebrio molitor* (L.) J. Protozool 14, 539–547.

Harry, O.G., 1970. Gregarina: their effect on the growth of the desert locust (*Schistocerca gregaria*). Nature 225, 964–966.

Kozloff, E.N., 1953. The morphogenesis of *Pyxinia crystalligera* frenzel, a gregarine parasite of *Dermestes maculatus* fabr. J. Morphol. 92, 39–77.

Lange, C., Lord, J., 2011. Entomopathogenic protists. In: Vega, Kaya (Eds.), Insect Pathology, second ed. Elsevier, San Diego, pp. 367–394.

Lantova, L., Ghosh, K., Svobodova, M., Braig, H.R., Rowton, E., Weina, P., Volf, P., Votypka, J., 2010. The life cycle and host specificity of *Psychodiella sergenti* n. sp. and *Ps. tobbi* n.sp. (Protozoa: Apicomplexa) in sand flies *Phlebotomus sergenti* and *Ph. tobbi* (Diptera: Psychodidae). J. Invertebr. Pathol. 105, 182–189.

Lantova, L., Svobodova, M., Volf, P., 2011. Effects of *Psychodiella sergenti* (Apicomplexa, Eugregarinorida) on its natural host *Phlebotomus sergenti* (Diptera, Psychodidae). J. Med. Entomol. 48, 985–990.

Rodriguez, Y., Omoto, C.K., Gomulkiewicz, R., 2007. Individual and population effects of eugregarine, *Gregarina niphandrodes* (Eugregarinida: Gregarinidae), on *Tenebrio molitor* (Coleoptera: Tenebrionidae). Environ. Entomol. 36, 689–693.

Schwalbe, C.P., Baker, J.E., 1978. Nutrient reserves in starving black carpet beetle larvae infected with the eugregarine *Pyxinia frenzeli*. J. Invertebr. Pathol. 28, 11–15.

Stanley, J., 1964. Washing the eggs of *Tribolium* for gregarine control. Can. J. Zool. 42, 920.

Sumner, R., 1936. Relationship of gregarines to growth and longevity in the mealworm, *Tenebrio molitor* L. Ann. Entomol. Soc. Am. 29, 645–648.

Votýpka, J., Lantová, L., Ghosh, K., Braig, H., Volf, P., 2009. Molecular characterization of gregarines from sand flies (Diptera: Psychodidae) and description of *Psychodiella* n. g. (Apicomplexa: Gregarinida). J. Eukaryot. Microbiol. 56, 583–588.