

# Strength in Numbers: Benefits of Group Living for the Control of Nematode Infection in the Dampwood Termite, *Zootermopsis angusticollis* Hagen

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## Abstract

The nesting, feeding and foraging ecology of termites places these social insects in contact with a diverse microbial community. Because termites live in colonies composed of thousands of individuals, the risk of infection among nestmates is significant. The selection pressures posed by entomopathogenic nematodes could have shaped many individual and social adaptations to resist parasitism. Here we show that group-living in the dampwood termite *Zootermopsis angusticollis* (Hagen) reduces susceptibility to infection by the nematode *Steinernema carpocapsae* (Weiser). Termite workers (pseudergates) were exposed to infective juveniles (IJs) of this entomoparasitic nematode and subsequently were maintained in groups or in isolation. Daily census were performed over a period of 35 days. Our results show that the probability of survival is significantly higher when termites live in a group setting and provide further support to the hypothesis that immunocompetence in *Z. angusticollis* is socially mediated.

## Introduction

Termites are an excellent model system to study social adaptations to resist disease and parasitism. Because of their nesting, feeding and foraging ecology, termites are continuously exposed to a variety of microorganism including potentially pathogenic and parasitic bacteria, fungi, viruses and nematodes (Rosengaus et al., 2003). The densely populated colonies together with the complex social organization of nestmates can further exacerbate the risks of infection within colonies. In spite of these costs, previous research has shown that group-living reduces the susceptibility of termites to infection by the fungus *Metarhizium anisopliae* (Rosengaus et al., 1998). The benefits of termite group-living in relation to the control of nematode infection, however, have not yet been studied.

The dampwood termite *Zootermopsis angusticollis* nests in moist, decayed wood which is usually surrounded and partially buried by soil. In such habitat, the risk of encountering soil nematodes may be particularly high. One such soil nematode is *Steinernema carpocapsae*, a generalist microscopic round worm which invades insect hosts through the mouth, anus and/or spiracles (Gaugler and Kaya, 1990). Once inside the host's hemocoel, the nematodes release their symbiotic bacteria, reproduce and feed off the host's internal organs, eventually causing death (Lewis et al., 1992). Upon emergence, the new infective juveniles (IJs) search for additional hosts to invade in order to start a new cycle of infection. If such emergence takes place within a termite colony, IJs should have no difficulty encountering new termite hosts. Thus, nematodes could pose important selection pressures on termites, both at the individual and colony levels.

## Aims

The purpose of this research was to study the costs and benefits of group-living in *Zootermopsis angusticollis* with respect to nematode infection. This basic information will increase our understanding of the behavioral, biochemical and physiological adaptations that termites have evolved to resist disease and parasitism. Furthermore, it will expand our knowledge of the role that social behavior and social interactions play on the incidence of parasitic infection in social insects.

## Figure 1

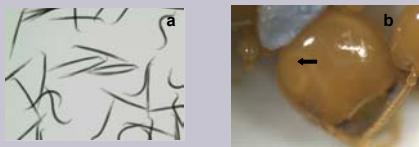


Fig. 1. *S. carpocapsae* infective juveniles (a) and nematode in head capsule of a *Z. angusticollis* nymph (b)

## Methods

**Host/parasite model species:** Infective juveniles of the nematode *Steinernema carpocapsae* (Mexican strain; Family Steinernematidae; Fig 1a) were chosen as the parasitic members of the experimental host/parasite association. Pseudergates (false workers) of the dampwood termite *Zootermopsis angusticollis* (Family Termitidae; Fig. 1b and 2) were used as the host model species.

## Figure 2

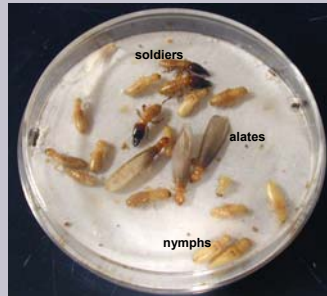


Fig. 2. The dampwood termite *Zootermopsis angusticollis*

## Methods

**Termite and nematode maintenance in the laboratory:** Stock termite colonies of *Z. angusticollis* were collected from Huddart Park, San Mateo County, California. *S. carpocapsae* (Mexican) was obtained from Dr. Khuong Nguyen (Department of Entomology and Nematology, University of Florida), and stocks were reared in wasp moth *Galleria mellonella* (L.) larvae (Webster's Waxie Ranch, Webster, WI). IJs were maintained in the laboratory at 15 °C using standard procedures (Stuart et al., 1996).

**Benefits of group living in the control of nematode infection:** To test whether group living enhances the survival of *Z. angusticollis*, we compared the susceptibility of infected and uninfected termites living in groups with that of infected and uninfected isolated nestmates. A total of 108 pseudergates (false workers) were used in this experiment, all originating from the same stock colony. Termites were assigned randomly to either the control (exposed to distilled water) or experimental treatments (exposed to IJs) and were maintained inside a plastic Petri dish (55 x 15 mm) lined with filter paper in groups composed of either 10 pseudergates (n = 60 individuals) or in isolation (n = 48 individuals). Control replicates were moistened with 300µl of distilled water while grouped experimental termites were exposed to 300µl of distilled water containing 600 IJs. To ensure that isolated termites were exposed to the same per capita dose of IJs as the grouped nestmates, isolated termites were exposed to 300µl of distilled water containing only 60 IJs. Survival was recorded daily for 35 days following the initial exposure. Dead termites were removed daily and placed in clean Petri dishes lined with moistened filter paper to confirm upon nematode emergence that *S. carpocapsae* was the cause of death. Corpses were also observed under a dissecting scope to verify that active IJs were indeed present in the host's hemocoel.

**Statistical analyses:** Several survival parameters were calculated, including the median survival time (LT<sub>50</sub>), percent survival at the end of the census period, the time course of survival (survival distributions); by Survival Analysis; SPSS), as well as the relative hazard ratios of death (Cox Proportional Regression Model, SPSS).

## Results

A Cox proportional regression model showed an overall significance in the susceptibility of the four treatments (Wald Statistic = 18.6, df = 3, P < 0.0001, Fig 3). Relative to the control/grouped treatment (the reference group given its lowest susceptibility), control termites maintained in isolation had 1.5 times the hazard ratio of death (Wald Statistic = 0.2, df = 1, P = 0.6). Grouped nestmates exposed to 600 IJs (corresponding to 60 IJs/termite) were 5.3 times more likely to die of nematode infection than control/grouped nestmates (Wald Statistic = 7.3, df = 1, P < 0.007). And isolated workers exposed to 60 IJs had a hazard ratio of death of 8.6 times that of the reference treatment (Wald Statistic = 12.4, df = 1, P < 0.0001). Additional survival parameters (LT<sub>50</sub>, median survival time and percent survival after 35 days post-exposure) corroborated the higher susceptibility of isolated termites relative to that of grouped nestmates, even after controlling for the number of IJs/termite (Table 1 and Fig 3).

Table 1. Survival parameters of termites in the four treatments.

Treatment	N	% Survival	LT <sub>50</sub>
control/grouped	20	85.0	> 35 <sup>a</sup>
control/isolation	14	78.6	> 35 <sup>a</sup>
exposed/grouped	40	42.5	29 ± 3 <sup>b</sup>
exposed/isolation	34	26.5	18 ± 4 <sup>b</sup>

The overall comparison among the four survival distributions was significantly different (Breslow Statistic = 25.2, df = 3, P < 0.0001; Kaplan-Meier Survival Test). \* and † indicate significant pairwise comparisons between each treatment and the control/grouped treatment. Due to multiple comparisons, a Bonferroni correction set the P at a more conservative threshold of P < 0.008. These pairwise comparisons should be viewed in conjunction with Fig. 1 and the relative hazard ratios of death (above).

### Confirmation rates:

The majority of termites exposed to IJs confirmed for *S. carpocapsae* infection. Eighty percent of the isolated exposed termites and 91% of the group exposed termites confirmed nematode infection. None of the control grouped and control isolated termites confirmed for *S. carpocapsae* infection.

Our results indicate that nematode exposed termites, regardless of whether they were maintained in groups or in isolation, have lower survival than control nestmates (Fig. 3 and Table 1). Yet within the nematode exposed treatments, termites maintained in groups (■ in Fig. 3) had a significantly lower susceptibility than isolated nestmates (● in Fig. 3).

## Discussion

Termites, as well as other social insects, live under important pathogenic and parasitic selection pressures (Schmid-Hempel, 1998; Rosengaus et al., 2003). Yet, through the coevolutionary history with their microbial community, termite hosts have evolved adaptations to reduce and/or resist microbial infection, including behavioral, biochemical and immunological responses (Rosengaus et al., 1998, 1999a,b, 2000a,b, 2004). Although previous research has shown that group-living does indeed reduce the susceptibility of nestmates (Rosengaus et al., 1998, Hughes et al., 2002; Traniello et al., 2002), to the best of our knowledge, this is the first time that the social mediation of nematode infection has been studied in termites. The observed lower susceptibility of grouped termites may be the result of important changes in the behavior of *Z. angusticollis*. Relative to their baseline behavior, nymphs and soldiers exposed to IJs of *S. carpocapsae* significantly increased the frequency and duration allotted to allogrooming (Wilson-Rich et al., in preparation). A similar response has also been reported when termites are exposed to fungal conidia (Rosengaus et al., 1998, 2000). Thus, in contrast to self-grooming, mutual grooming appears to be a very effective mechanism in the reduction of the incidence of fungal and nematode infection.

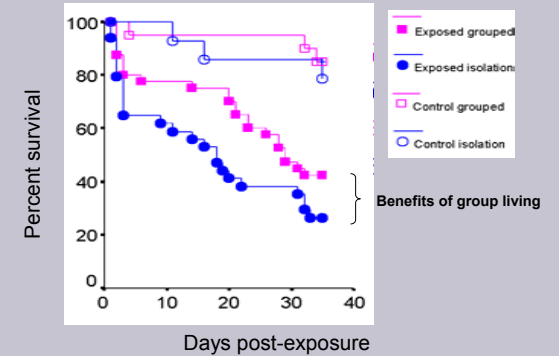


Fig. 3 Survival distributions of *Z. angusticollis* following exposure to water (control) or 60 IJs/termite and maintained either in isolation or in groups of 10 termites. The curves indicate the time course of survival and there are only one of the survival parameters measured. Results of the additional survival parameters are described in Table 1.

## Conclusion

Our results show that *Z. angusticollis* is susceptible to infection by *S. carpocapsae* and that termites living in a group setting have an increased probability of survival. Thus, parasitism by nematodes may be socially mediated in termites and perhaps other social insects. Our results, together with previous research, indicate that the benefits of group-living in social insects outweigh the potential costs of higher risks of infection. Understanding the basic pathology of termites to entomoparasitic nematodes will facilitate future studies focusing on the costs and benefits of group living in termites with respect to disease and disease resistance.

## Acknowledgement

We thank the administration of the Huddart Park in San Mateo County, California for allowing the collection of termite colonies. We also thank the Gann Academy-New Jewish High School for providing a flexible schedule and for their financial support to attend this meeting. This research was also supported by an RSDf grant issued by Northeastern University to Rebeca B. Rosengaus.

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