

Effect of mono- and polygyne social forms on transmission and spread of a microsporidium in fire ant populations

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Abstract

Thelohanian solenopsae is a pathogen of the red imported fire ant, *Solenopsis invicta*, which debilitates queens and eventually causes the demise of colonies. Reductions of infected field populations signify its potential usefulness as a biological control agent. *Thelohanian solenopsae* can be transmitted by introducing infected brood into a colony. The social forms of the fire ant, that is, monogyny (single queen per colony) or polygyny (multiple queens per colony), are associated with different behaviors, such as territoriality, that affect the degree of intercolony brood transfer. *T. solenopsae* was found exclusively in polygyne colonies in Florida. Non-synchronous infections of queens and transovarial transmission favor the persistence and probability of detecting infections in polygynous colonies. However, queens or alates with the monogyne genotype can be infected, and infections in monogyne field colonies have been reported from Louisiana and Argentina. Limited independent colony-founding capability and shorter dispersal of alate queens with the polygyne genotype relative to monogyne alates may facilitate the maintenance of infections in local polygynous populations. Demise of infected monogyne colonies can be twice as fast as in polygyne colonies and favors the pathogen's persistence in polygyne fire ant populations. The social form of the fire ant reflects different physiological and behavioral aspects of the queen and colony that will impact *T. solenopsae* spread and ultimate usefulness for biological control.

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1. Historical background of *Thelohanian solenopsae*

Thelohanian solenopsae Knell, Allen, and Hazard is a microsporidian pathogen of fire ants that was first discovered in 1973 from alcohol-preserved specimens of red imported fire ants, *Solenopsis invicta*, collected in Brazil (Allen and Buren, 1974). *T. solenopsae* was later described in detail by Knell et al. (1977). *Thelohanian solenopsae* also has been found in the congeners, *Solenopsis richteri* (Allen and Silveira-Guido, 1974), *S. saevissima*, *S. quinquecupis*, *S. macdonaghi*, and *S. blumi* [= *S. quinquecupis*] (Allen and Knell, 1980). However, infections were not detected in nine other non-*Solenopsis* genera of ants (Williams et al., 1998; Briano et al.,

2002). It was noted that *T. solenopsae*-infected field colonies of *Solenopsis* spp. could not be established in the laboratory and there seemed to be dramatic population reductions after 1–2 years in infected areas (Allen and Knell, 1980). In 1996, *T. solenopsae* was found in the United States (Williams et al., 1998, 2003), and rekindled interest in the pathogen as a potential biological control agent for fire ants. Laboratory studies documented 88–100% reduction in brood or the demise of *T. solenopsae*-infected *S. invicta* colonies in 6–12 months (Williams et al., 1999; Oi and Williams, 2002).

The impact of *T. solenopsae* on field populations of fire ants also has been documented. Briano et al. (1995a) reported an 83% reduction in populations of *S. richteri* in Argentina. In Florida, Oi and Williams (2002) reported fluctuating reductions of *S. invicta* populations which reached a maximum reduction of 63%. Cook (2002) reported colonies with *T. solenopsae* to have nest sizes, or

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mound volumes, that were 2.5 times smaller than uninfected colonies in Texas. In addition, colonies with *T. solenopsae* were more susceptible to fire ant bait containing hydramethylnon (Valles and Pereira, 2003) and infection by the entomopathogenic fungus *Beauveria bassiana* (Brinkman and Gardner, 2000). The detrimental impact of *T. solenopsae* on fire ants in both laboratory and field studies indicate that it may be useful as a biological control for fire ants.

2. Attributes and transmission of *T. solenopsae*

One of the most desirable attributes of *T. solenopsae* is that it infects and debilitates fire ant queens. Lower queen weights and premature death has been reported for queens from infected colonies (Knell et al., 1977; Williams et al., 1999). Briano et al. (1996) observed vegetative stages of *T. solenopsae* in *S. richteri* eggs and Valles et al. (2002) detected *T. solenopsae* in *S. invicta* eggs via PCR thus providing evidence for transovarial transmission.

Thelohania solenopsae infections can be initiated artificially in uninfected *S. invicta* colonies by introducing live, infected brood (Williams et al., 1999; Oi et al., 2001; Oi and Williams, 2002). Inoculations utilizing infected larvae resulted in an efficient, 80% colony infection rate, where infections were detected in queens and/or their offspring (Oi et al., 2001). Because infected brood can be used for inoculations, natural mechanisms of brood transfer between colonies can potentially result in intercolony transmission. Female fire ant reproductives initiating nuptial flights from infected colonies had infection rates of 75% (Oi and Williams, 2003). Infected newly mated queens have also been collected (Cook et al., 2003), and infected, incipient colonies have been reared from naturally infected newly mated queens (Oi and Williams, 2003). It has been hypothesized that infected incipient colonies and small colonies, that originate from infected newly mated queens, could be sources of inocula for the intercolony transmission of *T. solenopsae*. The transfer of inocula (i.e. infected brood) would occur by brood raiding, where incipient colonies steal brood from other incipient colonies (Tschinkel, 1992), or by colony raiding, where larger colonies overrun smaller colonies (Tschinkel, 1993). It has been demonstrated in the laboratory that colony raiding can result in *T. solenopsae* transmission (Oi and Williams, 2003). However, direct observation of transmission among colonies by brood or colony raiding in the field has not been documented.

Four spore types have been associated with *T. solenopsae* infections and are listed in Table 1 (Knell et al., 1977; Oi et al., 2001; Shapiro et al., 2003; Sokolova and Fuxa, 2001; Sokolova et al., 2003, 2004). The functions of these different spore types are currently under investigation. Sokolova et al. (2004) found binucleate megaspores in muscle and fat tissue surrounding ovaries, as well as in one oocyte. Large assemblages of megaspores, occasionally in close proximity to ovarioles, and the greater prevalence of megaspores in reproductive females than in other castes suggested a role in transovarial transmission. In addition, they state that the binucleate “*Nosema*-like” spores are involved in the autoinfection of adjacent fat body cells, eventually resulting in the production of octospores, and/or megaspores (Sokolova et al., 2004, 2005). Spores extracted from *T. solenopsae*-infected pupae (binucleate spore) and adults (most likely mixtures of spore types), and incorporated into various substrates consumed by fire ants, for example, hard-boiled chicken egg yolk, failed to result in transmission (Allen and Knell, 1980; Oi et al., 2001; Shapiro et al., 2003). Horizontal transmission has been reported when brood tended by infected adult workers were infected when they developed into adults (Allen and Knell, 1980; Oi et al., 2001). Chen et al. (2004) observed a preponderance of free (*Nosema*-like) spores, octospores, and megaspores in meconia shed from pupating larvae and hypothesized that this is a source of spores for the horizontal transmission of *T. solenopsae*. However, transfer of isolated spores, either alone or in combination, has not resulted in transmission. Thus, the spore type(s) involved in horizontal transmission of *T. solenopsae* is not known.

Of particular interest is the mechanism of *T. solenopsae* transmission to fire ant queens, as their demise is the most efficient method of eliminating colonies. Adult *S. invicta* workers possess a buccal tube lined with hairs and an infra-buccal pocket that forms an efficient filtering system that sieves out solid particles from ingested liquids (Fig. 1). Virtually all particle sizes greater than 0.75 μm in diameter were filtered out by minor sized workers, and for media and major workers, nearly all workers filtered 0.5 μm sized particles (Glancey et al., 1981; Petti, 1998). In a preliminary study examining virgin female reproductives, particles were not completely filtered out at 0.5 μm by reproductives alone, and when workers presumably would feed reproductives, not all 0.2 μm particles were filtered out (D.H. Oi, unpublished data). All known spore types of *T. solenopsae* are considerably larger than 0.75 μm and theoretically could not be ingested by adult *S. invicta* (Table 1). In

Table 1

Thelohania solenopsae spore types, size dimensions, and host developmental stage and castes of fire ants where spores were observed

Spore type	Size (μm)	Host stages and castes	Reference
Uninucleate meiospore (octospore)	3.3 \times 1.95	Adult workers, queens, alates	Knell et al. (1977)
Binucleate-free or <i>Nosema</i> -like spore	4.9 \times 1.85	Larvae, pupae, adult workers, and female reproductives	Knell et al. (1977)
Binucleate spore	4.5 \times 2.3	Pupae	Shapiro et al. (2003)
Binucleate megaspore	7.2 \times 3.8	Larvae, pupae, adult workers, and female reproductives	Sokolova and Fuxa (2001) and Sokolova et al. (2004)

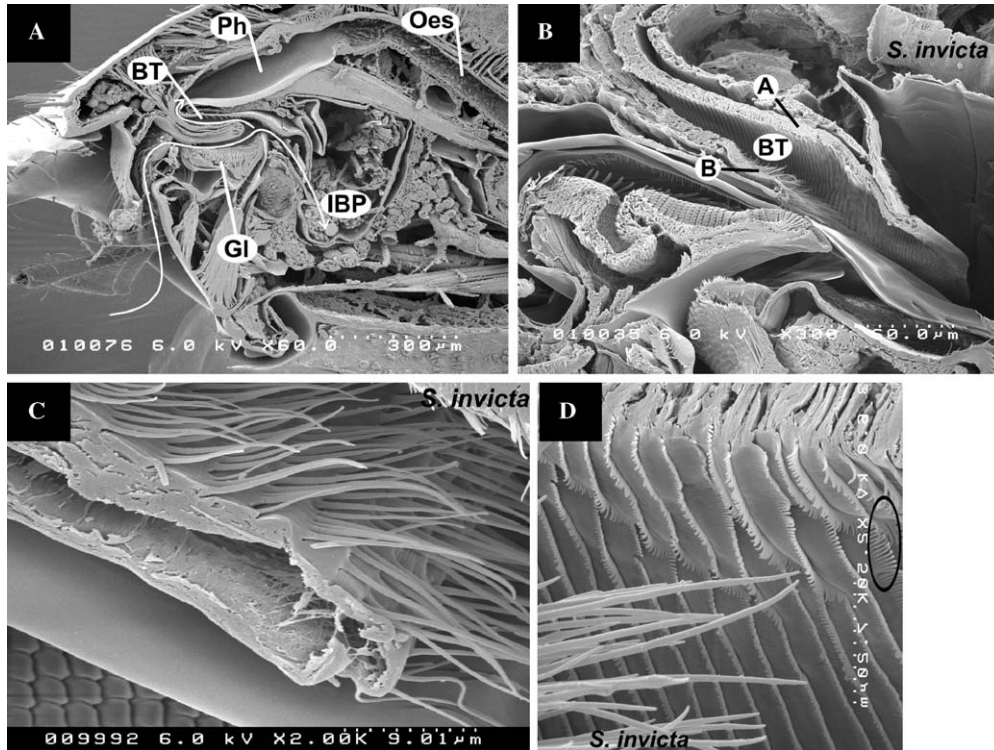


Fig. 1. (A) Sagittal section of the oral area of a *S. invicta* worker. Curved white lines denote pathway of ingested solids and liquids to infrabuccal pocket and buccal tube. (B) Buccal tube lined with filtering setae. Buccal tube is recurved in image thus “A” denotes ventral surface and “B” denotes dorsal surface. (C) Long blade-like dorsal setae of buccal tube. (D) Short ventral setae of buccal tube. BT, buccal tube; GI, glossa; IBP, infrabuccal pocket; Oes, esophagus; Ph, pharynx (specimens and images prepared by J. Petti).

contrast, maximum particle sizes of $45.8\mu\text{m}$ were ingested by *S. invicta* fourth instar larvae; apparently limited by the diameter of the esophagus (Glancey et al., 1981). Thus, the mechanism of horizontal transmission of *T. solenopsae* based on spore ingestion is an enigma.

3. Prevalence among fire ant social forms

There are two types of social forms of *S. invicta*. One form is comprised colonies with only a single, fertile queen, or monogyne colonies, and the other form has colonies with multiple fertile queens, or polygyne colonies. Monogyne colonies are territorial and as a result, fight with other *S. invicta* colonies. Because of this intercolony agonistic behavior, monogyne nests are farther apart with densities of 99–370 nests per hectare, with 100,000–240,000 ants per colony. In contrast, polygyne colonies are not antagonistic to other polygyne colonies and thus queens, workers, and immature ants (brood) can move between nests. Polygyne nests are usually smaller in size, closer together with densities of $494\text{--}1976\text{ ha}^{-1}$, and contain 100,000–500,000 ants per mature colony. In general, polygyne populations contain nearly twice the number of worker ants (35 million versus 18 million per ha) and biomass per unit area than monogyne populations (Macom and Porter, 1996). However, monogynous *S. invicta* are the predominant form in the United States (Porter et al., 1992). Distinguishing between the monogyne and polygyne colonies without locating fer-

tile queens can now be accomplished through molecular markers (Valles and Porter, 2003). In the United States, polygyne *S. invicta* colonies contain individuals with both *Gp-9^B* and *Gp-9^b* alleles (genotypes: *Gp-9^{BB}*, *Gp-9^{Bb}*, *Gp-9^{bb}*), with monogyne ants exhibiting only the *Gp-9^B* allele (Krieger and Ross, 2002).

Inoculations of *T. solenopsae* in 10 southern United States resulted in sustained infections only in *S. invicta* populations that were probably polygyne (D.H. Oi, unpublished data) based on colony density (Macom and Porter, 1996) and adult worker size (Greenberg et al., 1985, 1992). In addition, surveys for natural infections of *T. solenopsae* also indicated that infected colonies would typically be found in polygyne *S. invicta* populations (R.M. Pereira and D.H. Oi, unpublished data; Cook et al., 2003). To confirm this observation, a formal survey of *T. solenopsae* prevalence and *S. invicta* social form was conducted in three Florida pastures with varying ratios of monogyne to polygyne colonies (Fig. 2). *Thelohania solenopsae* was not detected in any of the sampled monogyne colonies while 83% of the polygyne colonies were infected (Oi et al., 2004). These results indicated that natural *T. solenopsae* infections in established field *S. invicta* colonies are only found in the polygyne social form despite the close proximity of monogyne colonies. However, the monogyne genotype does not preclude *T. solenopsae* infections. Infected alates with the monogyne genotype have been collected and colonies were reared from monogyne, newly mated queens that were

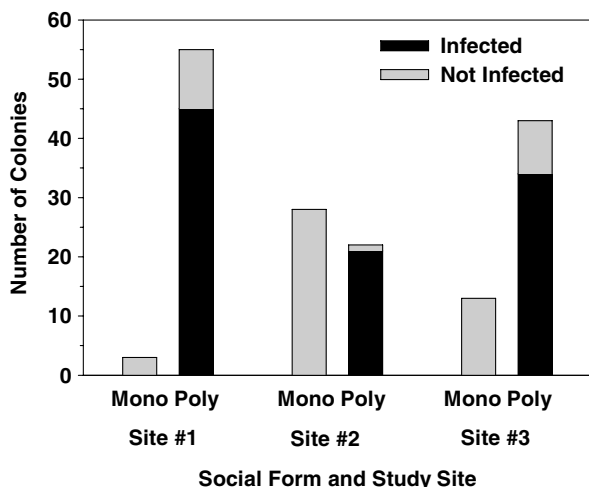


Fig. 2. Number of *T. solenopsae*-infected and uninfected *S. invicta* colonies between monogyne and polygyne social forms among three study sites in Florida.

naturally infected with *T. solenopsae* (Oi et al., 2004). Furthermore, *T. solenopsae* has recently been confirmed in monogyne field colonies in South America (Valles and Briano, 2004). Also, Fuxa et al. (2005a,b) reported *T. solenopsae* infections in monogyne colonies in Louisiana. However, the infections in the monogyne populations were localized and/or not sustained.

4. Effect of social form on persistence and spread

The high prevalence of *T. solenopsae* in polygynous *S. invicta* populations in the United States may be attributed to the presence of several queens per colony which can prolong the survivorship of colonies and the maintenance of infection. Infections initiated in laboratory, polygyne, *S. invicta* colonies survived (i.e. <90% brood reduction) an average of 11 months in contrast to 5 months for monogyne colonies (Williams et al., 1999; Oi and Williams, 2002). The slower demise of polygyne colonies could be due to the asynchronous infection of queens resulting in staggered declines of brood production and queen death. Infection rates of queens from field collected polygyne colonies ranged from 25 to 75% per *S. invicta* colony and 25 to 100% per *S. richteri* colony originating from Argentina (Oi and Williams, 2002). Laboratory colonies are confined and isolated, and without the addition of new, uninfected queens, all queens eventually become infected and the colony dies. In contrast, queens can be adopted by polygyne colonies (Glancey and Lofgren, 1988; Vander Meer and Porter, 2001), potentially extending colony survivorship and the presence of *T. solenopsae* infection.

Sources of queens include recently mated female reproductives after a nuptial flight (newly mated queens), and fertile ovipositing queens from established colonies (established queens). However, the successful adoption of queens is influenced by the social form of the queen and the adopting colony. Polygyne (*Gp-9^{Bb}*) newly mated queens had a

50% acceptance rate when introduced into polygyne colonies (Ross and Keller, 1998), and established queens with the *Gp-9^{Bb}* genotype were all accepted (Ross and Keller, 1998) or partially (72%) accepted (Vander Meer and Porter, 2001). In contrast, monogyne queens (*Gp-9^{BB}*), either newly mated or established, were not adopted into monogyne or polygyne colonies that already contained queens (Ross and Keller, 1998; Vander Meer and Porter, 2001).

As stated previously, *T. solenopsae* infections can also be initiated by introducing live, infected brood into colonies. One possible source of inocula is brood from infected incipient or small colonies, founded by newly mated queens (Oi and Williams, 2003). The social form of these founding queens influences their dispersal, thus potentially affecting the spread of inocula. Table 2 lists characteristics of newly mated queens relative to social form genotype they possess. In general, newly mated queens with the monogyne genotype (*Gp-9^{BB}*) are larger, have greater dispersal ability, more suited to found colonies independently, and not likely to be adopted. While these traits favor the spread of monogyne colonies, colonies founded by *T. solenopsae*-infected newly mated queens, grow slowly and as a consequence, probably have a poorer survivorship (Oi and Williams, 2003). Poor survivorship coupled with minimal queen adoption, does not favor the natural spread and high prevalence of *T. solenopsae* into monogyne fire ant populations. Fuxa et al. (2005a) reported a natural occurrence of *T. solenopsae* in a monogyne population of *S. invicta*, which after reaching a 63% prevalence ($n=19$ colonies) the pathogen was not detected after 2 years. Polygyne colonies were also found at the study site of which *T. solenopsae* was detected in 9 of 10 colonies. The polygyne colonies and the pathogen also were not apparent by the second year, suggesting that perhaps *T. solenopsae*-infected polygyne colonies in a predominantly monogyne fire ant population may not persist. Infection in the monogyne population probably dissipated as infected colonies die relatively quickly precluding extensive natural spread (Williams et al., 1999; Oi et al., 2004). In contrast, *T. solenopsae* has been documented in polygynous fire ant populations for well over 2 years (Fig. 3; Williams et al., 1999; Oi and Williams, 2002; D.H. Oi, unpublished data).

Newly mated queens with the polygyne genotype (*Gp-9^{Bb}*) are smaller than monogyne newly mated queens

Table 2
Newly mated queen characteristics with monogyne or polygyne genotypes

Character	Monogyne (<i>Gp-9^{BB}</i>)	Polygyne (<i>Gp-9^{Bb}</i>) ^a
Fresh weight	Heavy (≥ 14 mg)	Intermediate (11–12 mg)
Dispersal	More vagile	Less vagile
Colony founding	Independent ^b	Group ^c
Social form of queens' source colony	Primarily monogyne some polygyne	Only polygyne
Adoption	Executed—not adopted	Some adopted

^a Queens with *Gp-9^{Bb}* do not survive.

^b Group founding can occur initially, however only one queen survives.

^c Group founding hypothesized.

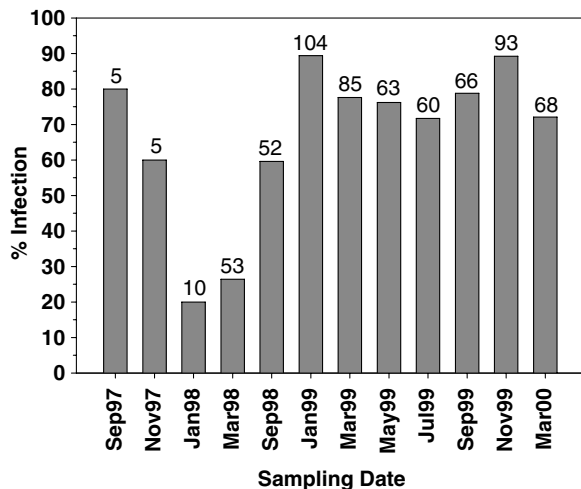


Fig. 3. Percentages of *T. solenopsae*-infected colonies from a polygynous, *S. invicta* field site in Alachua County, Florida. Numerals above bars indicate the number colonies examined per sample date.

(*Gp-9^{BB}*) and it is hypothesized that they would be more successful founding colonies in groups rather than independently as seen with the larger monogyne queens (Ross and Keller, 1998; DeHeer et al., 1999). Newly mated polygyne queens can have long or short mating flights (DeHeer et al., 1999) with the shorter flights facilitating potential adoption of these queens or their brood from incipient colonies by local polygyne colonies. Thus, if these queens were infected, the short dispersal would limit the spread of inocula most likely to polygynous fire ant populations. Polygynous fire ants have high nest densities (Macom and Porter, 1996) and are not territorial (Morel et al., 1990), thus allowing for interconnected nests and colonies (Bhatkar and Vinson, 1987). Hence, there is movement of workers, brood, and queens among established colonies and a greater potential for the transfer of inocula (Oi et al., 2001; Valles et al., 2002). These characteristics of a polygynous, *S. invicta* population allow for the spread *T. solenopsae* into uninfected colonies, or the spread of healthy ants into infected colonies, thus sustaining infections within a population.

5. Conclusion

The apparent limited natural dispersion of *T. solenopsae* into monogyne populations of fire ants in the United States represents a major obstacle to the biological control potential of this pathogen, as monogyny is the predominate social form of US fire ants. While natural spread of infection has been documented at the monogyne/polygyne interface, infections were not sustained. In contrast, *T. solenopsae*-infected, monogyne, *S. invicta* colonies in South America are apparently more common (Valles and Briano, 2004). Differences in the prevalence of infection between the US and South American social forms was speculatively attributed to differences associated with genetic founding bottlenecks, and perhaps differences in myrmecophiles that may serve as intermediate hosts (Valles and Briano, 2004). Isolates of

South American *T. solenopsae* with enhanced transmissibility is another possible reason for greater monogyne infection. The natural spread of *T. solenopsae* in polygynous fire ants populations is well documented and finding uninfected polygyne locations is becoming increasingly difficult (D.H. Oi, unpublished data). The effects of a preponderance of *T. solenopsae* infection throughout a majority of polygynous fire ant populations, such as a reduction in the availability of uninfected, polygyne newly mated queens on social form structure, remains to be seen. *Thelohania solenopsae* prevalence in surveys conducted in South America has ranged from 25 to 100% of the sites surveyed with 6–25% of the colonies sampled infected (Allen and Knell, 1980; Jouvenaz et al., 1980; Briano et al., 1995b). Improving the impact of *T. solenopsae* on fire ant populations in the United States may depend on enhancing horizontal transmission among monogyne populations, perhaps through bait formulations containing yet unknown infective spores small enough to bypass the filtering of the buccal tube or the establishment of more virulent isolates. Certainly, further studies on the life cycle and natural transmission of *T. solenopsae* would improve the basis to determine and hopefully utilize the full potential of *T. solenopsae* as a biological control of fire ants.

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