Prevalence of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) Infection in Monogyne and Polygyne Red Imported Fire Ants (Hymenoptera: Formicidae)

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ABSTRACT We determined the prevalence of natural field infections of the fire ant pathogen *Thelohania solenopsae* Knell, Allen, and Hazard in the monogyne and polygyne social forms of the red imported fire ant, *Solenopsis invicta* Buren, in three pastures in Florida. Social form was determined by examining the genotype of ants at the *Gp-9* locus. The monogyne form contains a single fertilized queen per colony, and colony members have a genotype of $Gp\text{-}9^{BB}$. In contrast, the polygyne form contains many fertilized queens per colony, and all three genotypes of $Gp\text{-}9^{BB}$, $Gp\text{-}9^{Bb}$, or $Gp\text{-}9^{bb}$ can be present within a colony. Among the study sites, ratios of monogyne:polygyne colonies ranged from 3:55–28:22, and infections rates were 42–78% when both social forms were included in the samples from each site. However, *T. solenopsae* infections were restricted to colonies of the polygynous social form. While *T. solenopsae* was only detected in polygynous colonies in the field, *T. solenopsae* infections were found in ants with the monogyne genotype. Ants from four colonies reared from field-collected, newly mated queens that were naturally infected with *T. solenopsae* were found to exhibit this genotype. *T. solenopsae* also was detected in individual alate female reproductives possessing the monogyne genotype, which were collected from polygynous colonies. Polygynous colonies can contain individual ants that possess either the polygyne or monogyne genotype. Thus, *T. solenopsae* infections can occur in fire ants with genotypes of either social form. Because making genotypic determinations of *S. invicta* social forms may be impractical in the field, we compared visual and genotypic determinations of polygyny and monogyny. Visual determinations, based mainly on the relative preponderance of major workers, corresponded to 85% of the genotypic determinations.

KEY WORDS pathogen, polygyny, *Solenopsis invicta*, fire ant, biological control

THE MICROSPORIDIAN PATHOGEN OF imported fire ants *Thelohania solenopsae* Knell, Allen, and Hazard was first observed in the red imported fire ant, *Solenopsis invicta* Buren, and the black imported fire ant, *Solenopsis richteri* Forel, from specimens collected in South America (Allen and Buren 1974, Allen and Silveira-Guido 1974). In 1996, Williams et al. (1998) collected *T. solenopsae*–infected *S. invicta* in Florida and it has since been collected in several other southern states in the United States (Williams et al. 1998, Cook 2002, D. H. O. unpublished data). Because *T. solenopsae*–infected queens have lower oviposition rates and die prematurely (Knell et al. 1977, Williams et al. 1999) and several studies have reported reductions in fire ant populations in infected field sites (Briano et al. 1995, Cook 2002, Oi and Williams 2002), there has been interest in trying to initiate *T. solenopsae* infections in uninfected areas (Williams et al. 1999). Inoculations of *T. solenopsae* in 10 states resulted in sustained infections only in *S. invicta* populations that were probably polygynous (D. H. O. unpublished data) based on colony density (Macom and Porter 1996) and adult worker size (Greenberg et al. 1985, 1992). Based on these characteristics, surveys for natural infections of *T. solenopsae* also indicated that infected colonies would typically be found in polygynous *S. invicta* populations (R. M. P. and D. H. O. unpublished data, Cook et al. 2003).

The identification of a single gene that is ostensibly responsible for monogyny or polygyny (Keller and Ross 1998, Ross and Keller 1998, Krieger and Ross 2002) led to development of a method that uses polymerase chain reaction (PCR) to determine the social form of *S. invicta* colonies (Valles and Porter 2003). Finding functional, or egg-laying, queens from field colonies can be a daunting task and may lead to erroneous results, especially when trying to confirm the presence of only a single queen (monogyny). With the PCR methodology, we were able to confidently assess the social form of numerous *S. invicta* colonies and thus determine if *T. solenopsae* infec-

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tion was more prevalent in polygyne populations located in Florida.

Materials and Methods

Infection in Field Colonies. *Solenopsis invicta* nests from three cattle pastures located in Alachua Co. (LaCrosse), Levy Co. (Williston), and Sumter (Lake Panasoffkee) Co., FL were sampled to determine the presence of *T. solenopsae* and whether they contained monogyne or polygyne colonies. In this study, nest refers to the physical location (often ground excavated by ants resulting in a mound of soil) that is inhabited by a colony of ants. Colony refers to a group of ants that typically contain brood, workers, and a queen(s). Sites were selected because they had a natural infection of *T. solenopsae* and there was a mixture of monogyne and polygyne *S. invicta.* At each pasture, adult worker caste ants were collected from all nests within circular plots of 0.025, 0.05, and 0.1 ha (1/16, 1/8, and 1/4 acres, respectively). Plot sizes were varied to maintain a reasonable number of samples that ranged from 12 to 53 nests/plot. Plot sizes for each site were as follows: Alachua Co., four 0.025-ha plots; Levy Co., one 0.025- and two 0.05-ha plots; Sumter Co., two 0.025-, one 0.05-, and one 0.1-ha plot.

Ants were collected by opening nests with a shovel and inserting a 7-ml plastic vial with inner sides coated with Fluon (Fluon-AD1; Asahi Glass Fluoropolymers USA, Chadds Ford, PA) to prevent ants that fell into the vial from escaping. Vials were retrieved within 45 min, kept cool, and frozen (−20°C) on returning to the laboratory. Samples were collected in November and December 2002. In general, field infections rates have been reported to be at their highest during the winter (Oi and Williams 2002). At the same time ants were collected, each colony was visually assessed as to being either monogyne or polygyne based on adult worker sizes (Greenberg et al. 1985, 1992). In general, our criteria for a designation of monogyny included the presence of a wider size range of adult workers with major workers readily apparent, whereas the adult workers in the polygyne colonies were predominately monomorphic minors, with only an occasional major worker observed. In conjunction with worker size, a colony would be designated polygyne if several dealate reproductive females were observed; however, dealates were not checked for insemination or oviposition.

Social form was determined with PCR by exploiting nucleotide substitutions between the *Gp-9* alleles to identify each allele ($Gp\text{-}9^B$, $Gp\text{-}9^b$) in *S. invicta* workers (Valles and Porter 2003). In the United States, polygynous *S. invicta* colonies contain individuals with both $Gp\text{-}9^B$ and $Gp\text{-}9^b$ alleles (genotypes: $Gp\text{-}9^{BB}$, $Gp\text{-}9^{Bb}$, $Gp\text{-}9^{bb}$), whereas monogyne colonies only have ants with $Gp\text{-}9^B$ alleles (Krieger and Ross 2002). Thus, from a homogenate of at least 20 workers per colony, polygyne samples would display, on an agarose gel, two bands corresponding to the $Gp\text{-}9^B$ and $Gp\text{-}9^b$ alleles. In contrast, monogyne samples would display a single band corresponding to the $Gp\text{-}9^B$ allele.

Thelohania solenopsae infection was determined by the PCR method described by Valles et al. (2002), which uses oligonucleotide primers specific to the *T. solenopsae 16S rRNA* gene. From the same colonies that social form was determined by PCR, another group of at least 20 workers per colony were homogenized to detect the presence or absence of infection.

The number of nests with infected and uninfected *S. invicta* was tested for independence from the type of social form (i.e., monogyne and polygyne) using the χ^2 test (SAS Institute 2001) over all locations. Visual assessment of colony polygyny and monogyny was compared with the PCR determination by χ^2 test of independence on the number of identical and conflicting results between the two methods (SAS Institute 2001).

Infection in Monogyne Laboratory Colonies. To determine if *T. solenopsae* infections could occur in monogyne *S. invicta* colonies, we tested four *T. solenopsae*–infected *S. invicta* laboratory colonies for the presence of the $Gp\text{-}9^B$ and $Gp\text{-}9^b$ alleles. The colonies were reared from naturally infected, individual, newly mated queens that were collected from four locations in Alachua Co., FL. A total of 130 of queens from these collections were examined for *T. solenopsae*, of which 12 were infected but only 4 were alive when social form determination by PCR was available. Rearing methods were adapted from Banks et al. (1981) and described by Oi and Williams (2003). *T. solenopsae* infection was confirmed by the presence of *T. solenopsae* meiospores in a single wet mount preparation per colony of a group of ≈30 macerated adult workers, which were examined under phase-contrast microscopy (Williams et al. 1999). The presence of *T. solenopsae* was reconfirmed using the PCR procedure described above.

***Gp-9* Alleles in Infected Alate Females.** To document the occurrence of infection in unmated female reproductives with either *Gp-9* allele, five *T. solenopsae*-infected, *S. invicta* colonies were excavated from a pasture in Levy Co., FL. All colonies were determined to be polygynous based on the visual criteria described earlier. Infection was confirmed by observing meiospores in worker ant samples. Four alate female reproductives per colony were individually tested for *T. solenopsae* infection and the *Gp-9* alleles using the PCR methodologies. Because the reproductives had not detached their wings within 24 h of collection, we assumed the reproductives had not mated, thereby precluding *Gp-9* allele introductions from sperm.

Results and Discussion

Infection in Field Colonies. Over all three study sites, *T. solenopsae* was not detected in any of the sampled monogyne colonies, whereas 83% of the polygyne colonies were infected. Thus, the rate of *T. solenopsae* infection was not independent of *S. invicta* social form ($\chi^2 = 93.96$, df = 1, $P < 0.001$). Because of an insufficient number of ants or inadequate DNA extraction, infection and/or social form could not be determined for 88 of 252 colonies. Ratios of monogyne

Table 1. Number of *T. solenopsae*–infected and total number of *S. invicta* colonies sampled[a] between monogyne and polygyne social forms among study sites in three Florida counties.

County	Social Form		Total
	Monogyne	Polygyne	
Alachua	0/3 (0.0)	45/55 (81.8)	45/58 (77.6)
Sumter	0/28 (0.0)	21/22 (95.5)	21/50 (42.0)
Levy	0/13 (0.0)	34/43 (79.1)	34/56 (60.7)
Total	0/44 (0.0)	100/120 (83.3)	100/164 (61.0)

Percentages of infected colonies are provided in parentheses.

[a] Infection and/or social form not determined for colonies from the following sites because of insufficient DNA or ants: Alachua, 43 of 101; Sumter, 14 of 64; Levy, 31 of 87 colonies.

to polygyne colonies varied among the study sites (Table 1). At the Alachua Co. site, we found only three monogyne colonies, all uninfected, despite a 78% *T. solenopsae* infection rate among both social forms combined. At this site, 82% of the 55 polygyne colonies were infected (Fig. 1). In Sumter Co., plots were located in two pastures in close proximity to each other (≈212 m apart, Fig. 2). Plots in one pasture contained only uninfected monogyne colonies, whereas the other pasture plots contained 22 polygyne colonies, of which 95% were infected, and there was also 1 uninfected monogyne colony. Between the two pastures in Sumter Co., 42% of the colonies were infected with *T. solenopsae.* The pasture in Levy Co. had a 61% infection rate, with 13 uninfected monogyne colonies, and a 79% infection rate among the polygynous colonies (Fig. 3). 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 and polygyne colonies. Fritz and Vander Meer (2003) also reported similar percentages of monogyne colonies within polygynous areas in north-central Florida.

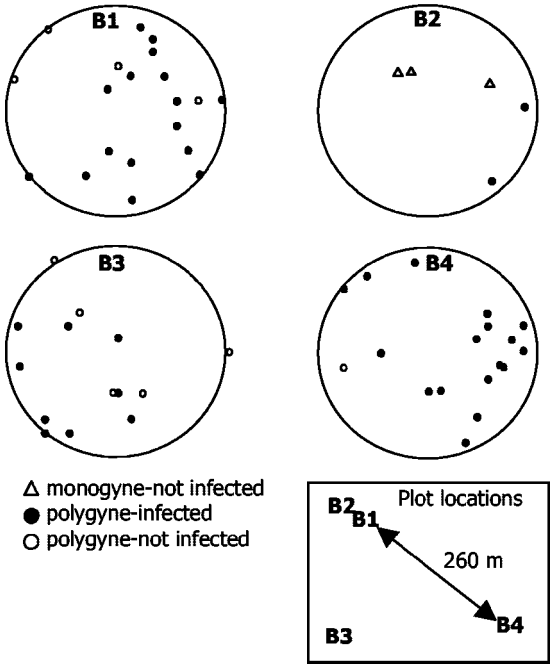

Fig. 1. Distribution of genotyped monogyne and polygyne colonies with and without *T. solenopsae* infection in four 0.025-ha plots located in a pasture in Alachua Co., FL (one infected polygyne colony is not shown in plot B1 because of missing latitude and longitude coordinates).

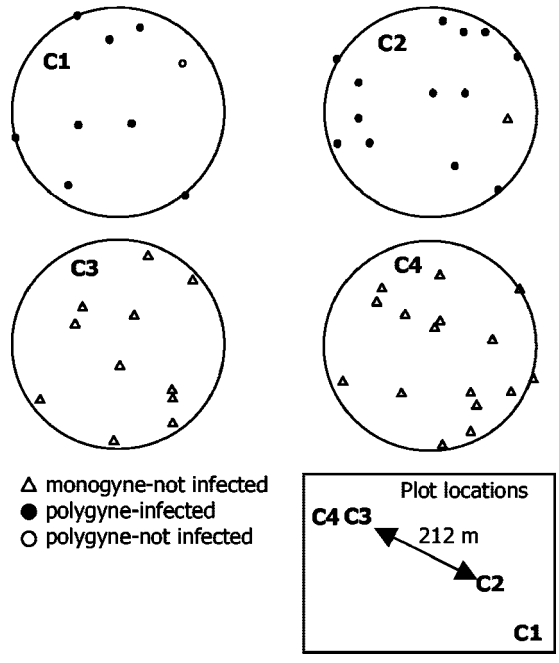

Fig. 2. Distribution of genotyped monogyne and polygyne colonies with and without *T. solenopsae* infection in four plots located in a pasture in Sumter Co., FL (plots C1 and C2 were 0.025 ha; plot C3 was 0.05 ha; plot C4 was 0.1 ha).

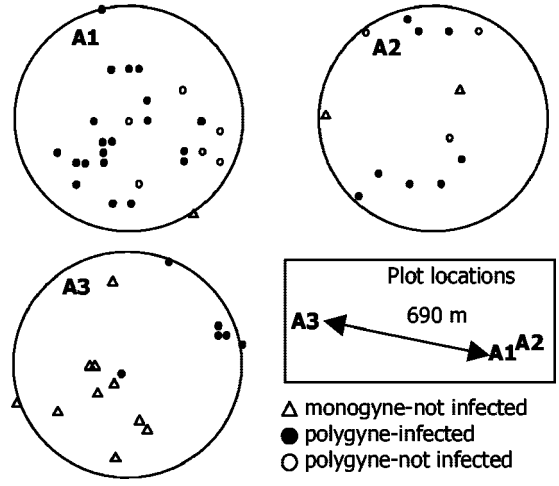

Fig. 3. Distribution of genotyped monogyne and polygyne colonies with and without *T. solenopsae* infection in three plots located in a pasture in Levy Co., FL (plots A1 and A3 were 0.05 ha; plot A2 was 0.025 ha).

There was significant agreement ($\chi^2 = 65.28$, df = 1, $P < 0.001$) between the visual assessment of monogyny and polygyny and the PCR determination, where 84.7% ($N = 163$) of the colonies had identical designations. For the conflicting assessments, 9.8% (16/163) of the colonies visually designated as monogyne were polygyne based on PCR, and 5.5% (9/163) of the colonies visually designated as polygyne were monogyne by PCR. Over one-half of the erroneous visual assessments occurred in the Alachua County site, where 55 of 58 colonies were polygyne. Various factors during sampling may affect the reliability of the visual assessments. For example, temperature extremes may cause brood tending minor workers to move deeper into nests than major workers, potentially resulting in the appearance of a greater preponderance of major workers in the sampled portion of the nest. Porter and Tschinkel (1993) have shown that *S. invicta* move within laboratory nests to reside at optimal temperatures and this can affect population assessments (Pranschke and Hooper-Bùi 2003). Incipient or young monogyne colonies have mostly minor workers, which could lead to an erroneous polygyne determination. The presence of uninseminated dealates also could cause erroneous designations of polygyne colonies. Morel et al. (1990) reported a 100% ($n = 16$) success rate of designating *S. invicta* social forms using a nestmate recognition bioassay, which was based on the degree of aggressive behavior exhibited between workers from different colonies. Among the methods of determining *S. invicta* social forms, functional queen isolation, worker size, nestmate recognition, and genotyping, the first two are most influenced by local sampling conditions. Nestmate recognition bioassays and genotyping can provide very accurate determinations of social form if performed correctly, but typically they require time consuming laboratory preparation/testing. Morel et al. (1990) needed a second series of tests to eliminate ambiguity of 12%. In our genotyping, 35% ($n = 252$) of our samples did not yield results because of difficulties in sample preparation or samples with inadequate numbers of ants. For a generalized appraisal of the type of social form at a study site, visual assessments of the relative preponderance of major *S. invicta* workers from field colonies can provide a reasonable (15% error rate) determination.

Infection in Monogyne Laboratory Colonies. In all four colonies reared from individual newly mated queens that were naturally infected with *T. solenopsae*, only the $Gp\text{-}9^B$ allele was detected. Thus, ants with the monogyne genotype ($Gp\text{-}9^{BB}$) can be infected with *T. solenopsae.* Furthermore, the failure to detect *T. solenopsae*-infected monogyne colonies in our field sites is unlikely to be attributed simply to the presence of the monogyne genotype.

***Gp-9* Alleles in Infected Alate Females.** *Thelohania solenopsae* infection was detected in 19 of the 20 female alates. Of the infected alates, 16 had both *Gp-9* alleles and three had only the $Gp\text{-}9^B$ allele. The single uninfected alate contained both *Gp-9* alleles. The three alates with the $Gp\text{-}9^{BB}$ genotype (i.e., monogyne) were collected from three different colonies. These results confirmed that *T. solenopsae* infection could occur in female alates exhibiting both genotypes ($Gp\text{-}9^{BB}$ and $Gp\text{-}9^{Bb}$).

Polygynous colonies can contain female alates that are homozygous or heterozygous at the *Gp-9* locus (Ross 1997, DeHeer et al. 1999). Queens and workers homozygous for the $Gp\text{-}9^b$ allele are supposedly selected against and are rare (Ross 1997). A majority of males from polygynous colonies are diploid and sterile (Hung et al. 1974, Ross and Fletcher 1985); thus, queens are most likely inseminated by males from monogyne colonies that carry only the $Gp\text{-}9^B$ allele. Ross (1997) estimated that 80% of 60 queens collected from polygyne colonies had mated with monogyne males. DeHeer et al. (1999) showed that $Gp\text{-}9^{BB}$ queens from polygynous colonies have a strong dispersal capability and suggested that they may be able to independently found new colonies. Thus, it is possible that the *T. solenopsae*–infected colonies that we reared from newly mated queens were derived from infected polygynous colonies, where female alates possessing $Gp\text{-}9^{BB}$ mated with $Gp\text{-}9^B$ males. Monogyne colonies are found in polygynous areas of north-central Florida (Fritz and Vander Meer 2003) and it is very possible that such matings occurred in areas where we collected the infected newly mated queens. While male reproductives can be infected with *T. solenopsae* (Knell et al. 1977, Cook et al. 2003), reports of venereal transmission of microsporidia in insects are rare (Becnel and Andreadis 1999). Nevertheless, it is a possibility that the infection in the laboratory colonies originated from infected, fertile, $Gp\text{-}9^B$ males derived from infected polygyne colonies.

Because queens that are homozygous or heterozygous with respect to the *Gp-9* alleles can be infected with *T. solenopsae*, behavioral factors may be contributing to the preponderance of infections in polygyne colonies in the field. Intercolony transmission of *T. solenopsae* can occur through the transfer of live infected brood (Williams et al. 1999, Oi et al. 2001). Polygynous fire ants have high nest densities (Macom and Porter 1996) and are not territorial (Morel et al. 1990), presumably resulting in interconnected nests/colonies (Bhatkar and Vinson 1987) that allow for the movement of workers, brood, and queens among colonies. Thus, there is greater potential for the transfer of inocula through the movement of infected brood or infected queens (Valles et al. 2002) between infected and uninfected polygynous colonies. 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. In contrast, monogyne colonies are territorial, with little brood exchange between colonies, and are thus insulated from *T. solenopsae* infections except perhaps by brood raiding (Tschinkel 1992) or colony raiding and brood abduction (Hölldobler 1981, Tschinkel 1993) of smaller infected colonies. Infection of monogyne colonies by these methods may be unlikely because polygyne populations seem to be able to expand their

area of infestation at the expense of monogyne colonies (Greenberg et al. 1992).

When monogyne colonies are infected, the probability of detecting them may be lower because of their faster demise than polygyne colonies. Williams et al. (1999) reported significant size reductions in infected, single-queen laboratory colonies within 6 mo. Significant reductions in infected polygyne laboratory colonies took an average of 10 mo, presumably because all queens within a colony are not simultaneously infected (Oi and Williams 2002). In addition, naturally infected, genetically monogyne ($Gp\text{-}9^{BB}$), newly mated queens reared in the laboratory produced smaller colonies than similar uninfected queens (Oi and Williams 2003). These effects may be accentuated under field conditions, thus preventing successful establishment of *T. solenopsae*–infected monogyne colonies. For example, monogyne colonies founded by infected newly mated queens may have poorer survivorship because of their slower growth. Incipient colonies with high rates of worker production are more likely to survive the brood raiding period and adverse environmental conditions (Markin et al. 1973, Tschinkel and Howard 1983, Tschinkel 1992). Last, the longevity of infected polygyne colonies may be extended by adoptions of new queens (Glancey and Lofgren 1988).

In summary, our pasture data showed that natural *T. solenopsae* infections were restricted to polygyne *S. invicta* colonies. Infected ants with the monogyne genotype could be reared from naturally infected queens, but it is possible that such queens could have originated from infected polygyne colonies. Polygyne colonies have traits that are conducive for horizontal transmission between colonies and that permit extended survivorship after being infected. In contrast, monogyne colonies are insulated from infection, but if infected, they die relatively quickly, and this may preclude extensive natural spread. For the classical biological control of imported fire ants with *T. solenopsae*, natural spread among monogyne colonies, the predominating form of *S. invicta* in the United States, may be limited. Thus, an augmentative approach may be needed to enhance the effectiveness of this pathogen of imported fire ants.

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