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PRESENCE OF *THELOHANIA SOLENOPSAE* AND *VAIRIMORPHA INVICTAE* IN SOUTH AMERICAN POPULATIONS OF *SOLENOPSIS INVICTA*

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The microsporidia, *Thelohania solenopsae* (Knell et al. 1977) and *Vairimorpha invictae* (Jouvenaz & Ellis 1986) have been reported to be effective self-sustaining biological control agents against the fire ant, *Solenopsis invicta* (Williams et al. 1999; Briano & Williams 2002; Briano et al. 2002). *Thelohania solenopsae* is well established among North and South American *S. invicta* populations and causes declines in queen egg production, queen weight, and worker and queen survivorship (Williams et al. 1999; Oi & Williams 2002). *Solenopsis invicta* is found in 2 distinct social forms, polygyne and monogyne; polygyne colonies have multiple fertile queens, while monogyne colonies have only a single fertile queen. Recently, North American *T. solenopsae* infections were shown to be restricted to the polygynous social form of *S. invicta* (Oi et al. 2004). Despite sympatry and sampling in areas with a high incidence of *T. solenopsae* infection (up to 78%), no monogyne fire ant colonies were found to be infected. Would this social form-specific *T. solenopsae* infection be similarly restricted to polygynous *S. invicta* in South America? To address this question, we determined the social form of archived *T. solenopsae*- and *V. invictae*-infected *S. invicta* samples from Argentina and Paraguay.

Samples of *T. solenopsae*- (n = 20) and *V. invictae*-infected (n = 15) nests of *S. invicta* were collected from the provinces of Santa Fe and Corrientes in Argentina and from Paraguay from 1999 to 2003. Infections for each microsporidian parasite were determined in each sample by the observation of spores in wet mount preparations of macerated adult ants under a phase-contrast microscope (400×, Briano & Williams 2002). Genomic DNA was extracted from 20 to 30 adult ants as described by Valles et al. (2002).

Social form was determined with PCR by exploiting nucleotide differences between the 3 *Gp-9* alleles ($Gp\text{-}9^{B}$, $Gp\text{-}9^{b}$, $Gp\text{-}9^{b'}$) found in South American *S. invicta* (Krieger and Ross 2002) by the method described by Valles & Porter (2003). Briefly, monogyne individuals are homozygous $Gp\text{-}9^{BB}$, whereas polygyne individuals are heterozygous (either $Gp\text{-}9^{Bb}$ or $Gp\text{-}9^{Bb'}$). $Gp\text{-}9^{B}$-specific oligonucleotide primers corresponded to positions 1683-1703 (primer 26: 5'CTCGCCGATTCTAACGAAGGA) and 2167-2199 (primer 16: 5'ATGTATACTTTAAAGCATTCCTAATATTTTGTC). Oligonucleotide primers designed to amplify either $Gp\text{-}9^{b}$ or $Gp\text{-}9^{b'}$ corresponded to positions 1307-1334 (primer 24: 5'TGGAGCTGATTATGATGAAGAGAAAATA) and 1702-1729 (primer 25: 5'GCTGTTTTTAATTGCATTTCTTATGCAG).

Multiplex PCR was conducted by the hot start method in a PTC 100 thermal cycler (MJ Research, Waltham, MA) under the following optimized temperature regime: 1 cycle at 94°C for 2 min, then 35 cycles at 94°C for 15 sec, 55°C for 15 sec, and 68°C for 30 sec, followed by a final elongation step of 5 min at 68°C. The reaction was conducted in a 50 µl volume containing 2 mM $MgCl_2$, 200 µM dNTP mix, 1 unit of Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA), 0.4 µM of primers p24, p25, p26, and p16, and 1 µl of the genomic DNA preparation (50 to 500 ng). PCR products (12 µl) were separated on a 1% agarose gel and visualized by ethidium bromide staining. For all experiments, positive and negative controls were run alongside treatments.

Among the 20 *T. solenopsae*-infected nests evaluated by PCR, 45% were polygyne and 55% monogyne (Table 1). Similarly, 46% and 54% of *V. invictae*-infected nests were polygyne and monogyne, respectively (Table 2). Therefore, *T. solenopsae* is not restricted to the polygyne social form as in North American *S. invicta* sampled in Florida. Despite failing to detect the *T. solenopsae*-infection in established monogyne colonies in North America, Oi et al. (2004) did find the infection in newly-mated monogyne queens (hypothesized to originate from *T. solenopsae*-infected polygyne queens). Thus, they concluded that the monogyne genotype ($Gp\text{-}9^{BB}$) did not preclude infection by *T. solenopsae*. In light of our results, their conclusion is validated. However, the question remains, why is *T. solenopsae* infection not observed in field populations of monogynous *S. invicta* in North America?

It is well documented that the population bottleneck during founding resulted in significant intrinsic differences between North and South American *S. invicta* (Ross et al. 1993). For example, there are differences in the number of alleles at the *Gp-9* locus (Krieger & Ross 2002), loss of variation at the major sex-determining locus resulting in greater male sterility (Ross et al. 1993), differences in queen relatedness among polygyne colonies (Ross et al. 1996), and differences in the proportion of permanently unmated queens (Ross

TABLE 1. *T. solenopsae*-INFECTED *S. invicta* EVALUATED FOR SOCIAL FORM.

Collection date	Collection site	Social form
27 April 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 505.4 km	Monogyne
27 April 1999	Santa Fe, Argentina, Route 11, 624.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 624.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 624.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 600 km	Monogyne
27 April 1999	Santa Fe, Argentina, Route 11, 649.9 km	Monogyne
5 July 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
5 July 1999	Santa Fe, Argentina, Route 11, 490.8 km	Monogyne
5 July 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
5 July 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
24 April 2001	Santa Fe, Argentina, Route 11, 560 km	Monogyne
26 April 2001	Santa Fe, Argentina, Route 11, 560 km	Monogyne
19 January 2003	Paraguay, Route 5, 368 km	Monogyne
24 January 2003	Misiones, Argentina, Iguazu Airport	Monogyne
24 January 2003	Misiones, Argentina, Iguazu, Airport	Monogyne
10 April 2003	Misiones, Argentina, Route 12, 1445 km	Monogyne
10 April 2003	Misiones, Argentina, Route 12, 1445 km	Monogyne

et al. 1996). Therefore, there may be a genetic basis for the differences in *T. solenopsae* infection among North and South American monogyne *S. invicta*. However, it would seem equally plausible that an extrinsic factor was responsible for the observed difference. Specifically, an intermediate host for *T. solenopsae* may be required for infection of monogyne *S. invicta*. Only a fraction of the known natural enemies of *S. invicta* are present in its North American range (Porter et al. 1997). Furthermore, perhaps the intermediate host would not be required for transmissibility in the polygyne social form because of their unique behavioral characteristics (less aggressive and more accepting of conspecific queens); colony organization can influence pathogen transmission in social insects (Naug & Camazine 2002).

Now that we know *T. solenopsae* infects field monogyne colonies in Argentina, investigations to elucidate the life cycle of this pathogen should continue with the hope of discovering a method to initiate a self-sustaining infection in monogyne *S. invicta* in the United States. *Vairimorph invictae* was included in this study because its suitability for release as a natural enemy of *S. invicta* in North America currently is being evaluated in

TABLE 2. *V. invictae*-INFECTED *S. invicta* EVALUATED FOR SOCIAL FORM.

Collection date	Collection site	Social form
27 April 1999	Santa Fe, Argentina, Route 11, 490.8 km	Monogyne
27 April 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 490.8 km	Monogyne
27 April 1999	Santa Fe, Argentina, Route 11, 560 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 560 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 560 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 560 km	Monogyne
27 April 1999	Santa Fe, Argentina, Route 11, 560 km	Monogyne
5 July 1999	Santa Fe, Argentina, Route 11, 560 km	Monogyne
5 July 1999	Santa Fe, Argentina, Route 11, 578.2 km	Monogyne
5 July 1999	Santa Fe, Argentina, Route 11, 600 km	Monogyne
5 July 1999	Santa Fe, Argentina, Route 11, 600 km	Monogyne
24 April 2001	Santa Fe, Argentina, Route 11, 729 km	Polygyne
28 February 2003	Santa Fe, Correientes, Route 123, 205 km	Polygyne

quarantine at the USDA-ARS facility in Gainesville, Florida, and we wanted to determine whether both social forms were capable of being infected.

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Summary

Thelohania solenopsae- and *Vairimorpha invictae*-infected *Solenopsis invicta* from South America were genotyped at the *Gp-9* locus to determine their social form. Unlike counterparts in the United States, monogyne nests are infected with both microsporidia species in South America.

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