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Biological Control 54 (2010) 342–348

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Seasonal variation and the co-occurrence of four pathogens and a group of parasites among monogyne and polygyne fire ant colonies

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ARTICLE INFO

Article history:
Received 5 April 2010
Accepted 10 June 2010
Available online 25 June 2010

Keywords:
Solenopsis invicta virus
Red imported fire ant
Kneallhazia solenopsae
Pseudacteon spp.
Biocontrol

ABSTRACT

A year-long survey was conducted to determine the seasonality and co-occurrence of four pathogens and a group of parasites in colonies of the red imported fire ant, *Solenopsis invicta*, in north-central Florida. *Solenopsis invicta* colonies were sampled and examined for the presence of *Pseudacteon* spp. (*P. curvatus*, *P. tricuspis*, *P. obtusus*) parasitic phorid flies, a microsporidian pathogen (*Kneallhazia solenopsae*) and 3 *S. invicta* viruses (SINV-1, SINV-2, and SINV-3) by PCR or RT-PCR methods. In addition, the social form designation of each colony (single- or multiple-queen) was determined by genotyping worker ants at the *Gp-9* locus to determine if the pathogens or parasites were associated with monogyne or polygyne fire ant colonies. Seasonal variability was observed in the prevalence of all pathogens/parasites examined, with SINV-1, SINV-3, and *K. solenopsae* exhibiting pronounced seasonality. SINV-1 and *K. solenopsae* infections were most prevalent among colonies during warmer periods of the year, while SINV-3 was most prevalent during the cooler periods. As hypothesized, pathogens were found more commonly in polygyne colonies than in monogyne colonies. Infection comparisons by social form revealed higher infection rates of *K. solenopsae* and SINV-2 in polygyne colony samples compared with monogyne colony samples. The overall colony infection and parasitism rate among the 360 colonies sampled, regardless of social form, was 60.3% (SINV-1), 8.9% (SINV-2), 10.8% (SINV-3), 22.5% (*K. solenopsae*), and 8.1% (*Pseudacteon* flies). An interesting pattern was observed between the number of different pathogens or parasites detected in monogyne and polygyne colonies. The majority of monogyne colonies (>80%) were either uninfected or infected or parasitized with only a single pathogen/parasite while the majority of polygyne colonies (>55%) were infected or parasitized with 2 or more pathogens/parasites simultaneously. Higher pathogen/parasite prevalence among polygyne colonies is attributed to lower genetic relatedness (among nestmates), increased colony longevity, and the proclivity of polygyne colonies to share workers, brood and queens among their interconnected colonies. Evaluation of pairwise co-occurrence data indicated that the pathogens and fly parasites were usually independently distributed among host colonies; however, unknown local factors did cause several significant deviations from expected values.

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1. Introduction

Since its introduction at Mobile, Alabama, in the early 1930s (Lofgren et al., 1975), the red imported fire ant, *Solenopsis invicta* Buren, has spread and become thoroughly established throughout the southern states and has been found in recent years in parts of New Mexico, Arizona, and California (Williams et al., 2001). Although insecticides remain effective at controlling *S. invicta* in the United States, they must be used continuously to provide sustained control. If insecticide use is discontinued, fire ant populations quickly re-inhabit the previously treated area. Empirical and anecdotal evidence indicate that in regions where *S. invicta* is indigenous [e.g., Argentina (Caldera et al., 2008)], it is not considered a serious pest (Porter et al., 1992). This difference has been attributed to a lack of natural enemies in the introduced population of *S. invicta* allowing it to compete with native ants without the costs of most pathogens and parasites (Porter et al., 1997; Mitchell and Power, 2003). Indeed, *S. invicta* has a higher density and possesses larger mound volumes in the U.S. compared with South America (Porter et al., 1992, 1997).

Permanent sustainable control of *S. invicta* populations across its U.S. range will likely depend on self-sustaining biological control agents. Three major groups of natural enemies are currently found in the U.S. (Oi and Valles, 2009). These include a group of parasitic flies in the genus *Pseudacteon* (Diptera: Phoridae) intentionally introduced from South America (Porter, 1998; Callcott et al., submitted for publication), a microsporidian pathogen, *Kneallhazia solenopsae* Knell, Allen, and Hazard (Microsporidia: Burenellidae) (Williams et al., 1998), and 3 positive sense,

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1049-9644/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.biocontrol.2010.06.006

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single-strand RNA viruses, *S. invicta* virus 1 (SINV-1), SINV-2, and SINV-3 also likely from South America but of uncertain origins (Valles et al., 2004, 2007a; Valles and Hashimoto, 2009). Among the viruses, only SINV-1 has been officially classified into the *Dicistroviridae* (Buchen-Osmond, 2006). North Florida provides an ideal area for examining the prevalence and co-occurrence of these natural enemies because all of them are well established in this region.

Solenopsis invicta colonies exist in two distinct forms, polygyne and monogyne. Polygyne colonies contain more than one fertile queen and monogyne colonies contain a single fertile queen. This colony organization can have a significant influence on parasitism and infection (Schmid-Hempel, 1998). Specifically, it seems likely that interconnected polygyne supercolonies would be at greater risk of infections than monogyne colonies which do not normally share brood and workers among neighboring colonies. In fact, *K. solenopsae* infection rates of *S. invicta* have been reported to be significantly higher in polygyne colonies compared with monogyne colonies (Oi et al., 2004; Fuxa et al., 2005a,b). Understanding the possible propensity of certain pathogens and parasites to infect one social form (monogyny or polygyny) over the other is relevant to effective control of *S. invicta*. Therefore, to test the hypothesis that the SINV-1, SINV-2, SINV-3, *K. solenopsae*, and/or *Pseudacteon* flies exhibit a bias toward a particular *S. invicta* social form, we examined co-occurrence of pathogen/parasite infections in monogyne and polygyne *S. invicta* colonies across seasons in north-central Florida.

2. Materials and methods

2.1. Ant collections and nucleic acid preparation

Worker ants were collected by plunging a 20-ml glass scintillation vial into an established *S. invicta* nest. After sufficient numbers of ants (>30) had fallen into the vial, they were returned to the laboratory for nucleic acid extraction. RNA was extracted from a pooled group of 10 arbitrarily selected workers from each nest with Trizol reagent according to the manufacturer's directions. Next, DNA was extracted from another pooled group of 10 arbitrarily selected workers from each corresponding colony as described previously (Valles et al., 2002). Nucleic acid concentration was determined spectrophotometrically and adjusted to 50 ng/μl by further dilution with diethyl-pyrocarbonate-treated water (RNA) or TE (10 mM Tris, pH 8.0, 1 mM EDTA) buffer (DNA).

Ant collections were conducted monthly from September 2008 through August 2009, inclusive, from three locations in Gainesville, Florida, and surrounding cities. Ant samples were collected from 10 arbitrarily selected colonies from each collection site (initially about a 50-m diameter) per sampling date (Table 1). Two collection sites were permanent (i.e., revisited) each month and one site was from a new location (variable) each month. The two permanent sites were around our USDA research center (along Hull road and SW 23rd Drive; 29.63543, −82.35991) and Paynes Prairie (north side along US highway 441; 29.58308, −82.33883). The USDA site was predominately comprised of polygyne colonies (78.3%), while the Paynes Prairie site was predominately comprised of monogyne colonies (95.8%). Samples taken from the permanent sites were collected from ever-increasing areas (at each of the two locations) to minimize re-sampling the same ant nests. However, re-sampling from identical colonies was a possibility over the course of the year-long survey. The variable site was included without knowing the prevalence of colony social form *a priori*. On average, 85% of the colonies in the variable sites were monogyne, with five sites being completely monogyne. The remaining sites were a mixture of social forms that ranged from 40% to 90% monogyne.

2.2. Molecular assays

Molecular-based assays were used to examine each field-collected sample for the presence of SINV-1, SINV-2, SINV-3, *K. solenopsae*, *Pseudacteon* spp. and to determine the social form of the corresponding colony. The fire ant viruses (SINV-1, -2, and -3) were detected by multiplex RT-PCR using virus-specific oligonucleotide primers as described previously (Valles et al., 2009b). *Kneallhazia solenopsae* and *Pseudacteon* flies were detected by multiplex PCR of a DNA template (Valles et al., 2009a). The oligonucleotide primers used to detect the 18S region of the *Pseudacteon* rRNA gene were designed to amplify *P. curvatus* Borgmeier, *P. obtusus* Borgmeier, and *P. cultellatus* Borgmeier equally. Thus, the detection method was incapable of discerning which decapitating fly species was parasitizing the ant host. Colony social form was determined by conducting allele-specific PCR at the *Gp-9* locus (Valles and Porter, 2003). Homozygous ($Gp\text{-}9^{BB}$) samples were considered monogyne and heterozygous ($Gp\text{-}9^{Bb}$) samples polygyne (Krieger and Ross, 2002).

2.3. Statistical analysis

Seasonal prevalence data were presented by calculating the mean and standard error values for each pathogen using month as the classification variable and each site location as experimental unit ($n = 3$ locations per month, 10 colonies per location). Analysis of variance was conducted to determine whether pathogen prevalence was biased toward any particular collection site using Proc GLM and Scheffe's multiple comparison procedure to separate the means (SAS, 1988). Student's *t*-test was used to compare the proportion of monogyne and polygyne colonies at each site (and all sites combined) infected with each pathogen. In this case, the Satterthwaite method was used to determine whether the

Table 1
Summary of collection information for the variable sites within Florida.

Date collected	Location (street, city)	Latitude, longitude	% Polygyne collected
17 September 2008	Fred Bear Drive at State Road 24, Gainesville	29.61148, −82.38248	40
28 October 2008	Fred Bear Drive at State Road 121, Gainesville	29.60144, −82.37643	0
25 November 2008	State Road 26 at SW 226th Street, Gainesville	29.65449, −82.58100	0
23 December 2008	NW 43rd Street at US441, Gainesville	29.74105, −82.38887	10
27 January 2009	NW 43rd Street at NW 73rd Avenue, Gainesville	29.72257, −82.38883	10
17 February 2009	SW 23rd Street at SW 35th Place, Gainesville	29.62.28, −82.35587	20
13 March 2009	Williston Road at State Road 27, Gainesville	29.39563, −82.44724	0
16 April 2009	State Road 20 at SE 30th Street, Gainesville	29.64228, −82.28791	0
19 May 2009	Gator National Speedway, Gainesville	29.75945, −82.27704	0
24 June 2009	State Road 222 at NW 83rd Street, Gainesville	29.68842, −82.43007	10
14 July 2009	US Highway 441 at State Road 234, Micanopy	29.51133, −82.28416	30
20 August 2009	State Road 24 at US Highway 19, Otter Creek	29.32681, −82.77717	60

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variances between samples were unequal. Student's *t*-test was also used to compare the number of organisms co-occurring in monogyne and polygyne colonies after arcsine transformation of the data. Pairwise co-occurrence patterns of the four pathogens and the phorid flies in the colony samples, regardless of social form, were examined with Chi-square 2 by 2 contingency tables to determine if their co-occurrence patterns were higher or lower than expected by chance. The association of monthly mean temperature and rainfall data collected from the National Oceanic and Atmospheric Administration website (http://www.noaa.gov/) for Gainesville, Florida, with the prevalence of each pathogen and parasite in the survey were examined by Pearson's correlation (SAS, 1988).

3. Results

All four pathogens and the phorid flies were detected at both of the fixed sites (USDA and Paynes Prairie) and in the annual composite of the 12 variable sites; however, the relative abundances of these pathogens and parasites varied with season and by frequency of monogyne and polygyne colonies at each site. The overall colony infection rate among the 360 colonies sampled, regardless of social form, was 60.3% (SINV-1), 8.9% (SINV-2), 10.8% (SINV-3), 22.5% (*K. solenopsae*), and 8.1% (*Pseudacteon* flies).

No significant differences in the prevalence of SINV-1, SINV-3, or *Pseudacteon* flies were observed among sites (Table 2). However, the prevalence of *K. solenopsae* ($F = 10.1$; df = 2, 33; $p < 0.001$) and polygyny ($F = 84.1$; df = 2, 33; $p < 0.001$) were significantly greater at the USDA site. The prevalence of SINV-2 was significantly greater at the USDA site compared with the variable sites.

The number of *S. invicta* samples infected with SINV-1, -2, -3, *K. solenopsae*, and *Pseudacteon* flies varied during the year-long collection period (Fig. 1A and B). In general, the prevalence of SINV-1 in *S. invicta* colonies was highest during the spring and summer months (April through August) and lowest during the winter (January through March). All colony samples ($n = 30$) collected during June were infected with SINV-1. The SINV-1 infection rate correlated positively ($r = 0.92$, $p = 0.0001$) with average ambient air temperature (Table 3). In addition to temperature, SINV-1 prevalence and rainfall exhibited a significant correlation ($r = 0.59$, $p = 0.04$) (Table 3). The only other pathogen exhibiting a significant correlation was SINV-3 with temperature ($r = -0.59$, $p = 0.04$). In this case, a negative relationship was observed; as the average air temperature in the collection area decreased, SINV-3 prevalence among fire ant colonies increased. No significant correlations were observed between SINV-2, *Pseudacteon* spp., or *K. solenopsae* with rainfall or temperature (Table 3). However, *K. solenopsae* did show a fairly strong increasing trend with higher average temperature.

Analyzed without regard for social form, the infection rate of *K. solenopsae* among *S. invicta* colonies remained fairly low (<25%, Fig. 1B) in the cooler months from October through June. However, the infection rate increased above 30% during the summer period from June to September with a peak infection rate in August (60%). Correlation analysis (Table 3) showed an insignificant but trending relationship between temperature and *K. solenopsae* infection. When the *K. solenopsae* infection rate was examined by colony social form as the classification variable, polygyne colonies typically exhibited a higher infection rate of *K. solenopsae* than monogyne colonies collected from the same areas (Fig. 1B, inset). When the *K. solenopsae* infection rate was compared among all colony samples (by site) collected during the year-long survey (using each site as an experimental unit), *K. solenopsae* infection rate in polygyne colonies was significantly greater (Paynes Prairie, Variable, and combined sites) than monogyne colonies (Table 4). Monogyne colonies had a higher rate of *K. solenopsae* infection when they were collected from the USDA site where polygyne colonies were common (18% at the USDA site versus 7% at the Paynes

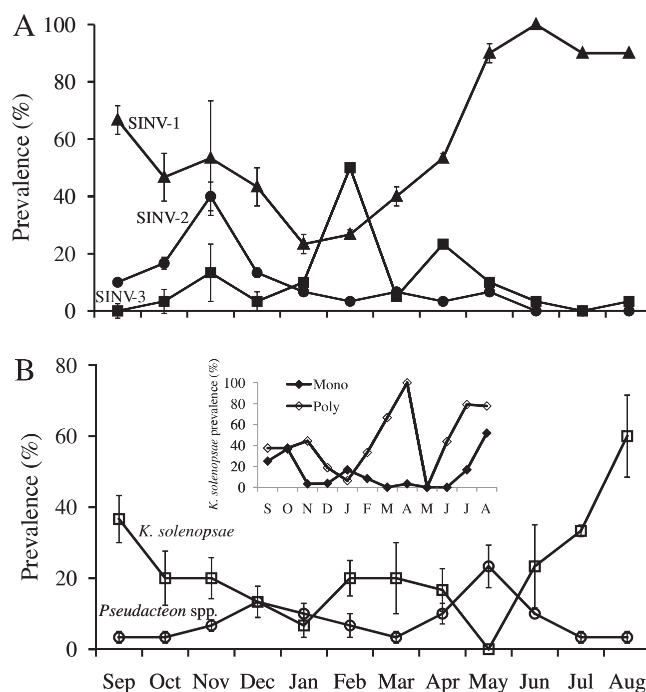

Fig. 1. (A) Prevalence of SINV-1 (▲), SINV-2 (●), and SINV-3 (■) at three collection sites in north-central Florida, during the period September 2008 through August 2009. Points represent the mean and standard error determined from 30 colonies. (B) Prevalence of *Kneallhazia solenopsae* (□) and *Pseudacteon* flies (○) at three collection sites in north-central Florida, during the period September 2008 through August 2009. Points represent the mean and standard error determined from 30 colonies. Inset: Prevalence of *K. solenopsae* during the same period in monogyne and polygyne colonies.

Table 2
Comparison of pathogens/parasites infecting *Solenopsis invicta* workers at three sites and the proportion of each social form found at each site and overall. Mean proportion (± standard error) of sampled colonies infected with each pathogen or parasite. Sample size is 12 in each case for the specific sites and 36 for sites combined. Means within a row with different letters are significantly different ($p < 0.05$) by Scheffe's multiple comparison procedure.

Parasite/Pathogen/Social form	Mean proportion at site (± SE, $n = 12$)			Grand mean proportion (± SE, $n = 36$)
	Paynes Prairie	USDA	Variable	
SINV-1	53.3 ± 10.2 A	70.8 ± 5.7 A	56.7 ± 10.4 A	60.3 ± 5.2
SINV-2	7.5 ± 4.3 AB	16.7 ± 4.3 A	2.5 ± 1.8 B	8.8 ± 2.3
SINV-3	11.7 ± 4.6 A	12.5 ± 3.7 A	8.3 ± 5.9 A	10.8 ± 2.7
Pseudacteon spp.	6.7 ± 2.3 A	8.3 ± 2.7 A	9.2 ± 3.8 A	8.1 ± 1.7
K. solenopsae	11.7 ± 6.4 B	44.2 ± 7.3 A	11.7 ± 3.2 B	22.5 ± 4.2
Polygyne	4.2 ± 2.6 B	78.3 ± 4.4 A	15.0 ± 5.6 B	32.5 ± 6.0
Monogyne	95.8 ± 2.6 A	21.7 ± 4.4 B	85.0 ± 5.6 A	67.5 ± 6.0

Solenopsis invicta viruses (SINV-1, SINV-2, and SINV-3); *Pseudacteon* spp. = *P. curvatus*, *P. obtusus*, and *P. cultellatus*; *K. solenopsae* = *Kneallhazia solenopsae*.

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Table 3
Relationships between the prevalence of each fire ant pathogen and average temperature and rainfall during the month in which collection occurred. Pearson correlation of zero between the pathogen infection rate and temperature or rainfall indicates that each variable has no linear predictive ability for the other (SAS, 1988).

Pathogen/Parasite	Variable[a]	Correlation coefficient (*r*)	Probability (*r* = 0)
SINV-1	Temperature	0.92	***0.0001***
	Rainfall	0.59	***0.04***
SINV-2	Temperature	−0.43	0.16
	Rainfall	−0.47	0.13
SINV-3	Temperature	−0.59	***0.04***
	Rainfall	−0.23	0.48
Pseudacteon spp.	Temperature	−0.09	0.77
	Rainfall	0.26	0.41
K. solenopsae	Temperature	0.55	0.06
	Rainfall	0.11	0.74

Solenopsis invicta viruses (SINV-1, SINV-2, and SINV-3); *Pseudacteon* spp. = *P. curvatus*, *P. obtusus*, and *P. cultellatus*; *K. solenopsae* = *Kneallhazia solenopsae.*
[a] Average monthly temperature or rainfall in Gainesville, Florida, during the month in which each collection sample was taken.

Table 4
Comparison of pathogens/parasites infecting *Solenopsis invicta* workers at three sites between social form (monogyne and polygyne). Mean proportion (± standard error) of sampled colonies infected with each pathogen or parasite are reported.

Pathogen/Parasite	Mean proportion at site (± SE)[a]							
	Paynes Prairie		USDA		Variable		All sites combined	
	Monogyne	Polygyne	Monogyne	Polygyne	Monogyne	Polygyne	Monogyne	Polygyne
None	24.3 ± 3.7	11.1 ± 11.1	15.2 ± 6.3	6.1 ± 1.9	31.0 ± 4.3*	3.5 ± 3.5	26.0 ± 2.6*	6.0 ± 1.7
SINV-1	44.1 ± 4.3	44.4 ± 17.6	51.5 ± 8.8	41.7 ± 3.9	44.0 ± 4.6	58.6 ± 9.3	44.9 ± 3.0	44.3 ± 3.5
SINV-2	6.6 ± 2.1*	ND	3.0 ± 3.0*	11.7 ± 2.5	1.7 ± 1.2	3.5 ± 3.5	4.2 ± 1.2*	10.0 ± 2.1
SINV-3	11.8 ± 2.8*	ND	6.1 ± 4.2	7.4 ± 2.1	6.9 ± 2.4	6.9 ± 4.8	9.1 ± 1.7	7.0 ± 1.8
Pseudacteon spp.	5.9 ± 2.0*	ND	6.1 ± 4.2	4.9 ± 1.7	9.5 ± 2.7	3.5 ± 3.5	7.4 ± 1.6	4.5 ± 1.5
K. solenopsae	7.4 ± 2.3*	44.4 ± 17.8	18.2 ± 6.8	28.2 ± 3.5	6.9 ± 2.4*	24.1 ± 8.1	8.4 ± 1.7*	28.4 ± 3.2

Solenopsis invicta viruses (SINV-1, SINV-2, and SINV-3); *Pseudacteon* spp. = *P. curvatus*, *P. obtusus*, and *P. cultellatus*; *K. solenopsae* = *Kneallhazia solenopsae.*
ND, not detected.
[a] Means indicated with an asterisk between social forms within a site are significantly different by Student's *t*-test ($p < 0.05$) using the Satterthwaite method to test for unequal variances.

Prairie and Variable sites; Table 4). We found that 74% of samples ($n = 108$, Paynes Prairie) and 61% of samples ($n = 77$, Variable Sites) were infected from monogyne sites where polygyne colonies were uncommon (⩽10%). By comparison, 81% of monogyne samples ($n = 26$, USDA) and 72% of monogyne samples ($n = 25$, variable sites) were infected where polygyne colonies were common.

The percentage of *S. invicta* samples parasitized by one of the *Pseudacteon* species ranged from 3.3% to a high in May, 2009, of 23% (Fig. 1B). When all sites were combined, *Pseudacteon* parasitism was not significantly different between polygyne and monogyne colonies (Table 4); the parasitism rate was 7.4% and 4.5% in monogyne and polygyne colonies, respectively.

When analyzed without regard for pathogen/parasite species or collection site, but rather, pathogen/parasite number (i.e., simultaneous infections), significant distinctions were again observed between polygyne and monogyne colonies (Fig. 2). A significantly greater proportion of monogyne colonies (30.9 ± 6.3%; $t = 2.9$; $p = 0.008$) were free of pathogens or parasites compared with polygyne colonies (10.2 ± 3.2%). Similarly, a significantly greater proportion of monogyne colonies (52.4 ± 5.3%; $t = 3.3$; $p = 0.003$) were parasitized by a single pathogen/parasite compared with polygyne colonies (31.4 ± 3.5%). Colonies parasitized with two or more organisms simultaneously were significantly greater ($t = -4.3$; $p = 0.003$, 2 pathogens; $t = -2.1$; $p = 0.047$, 3 pathogens) in polygyne than monogyne colonies. Thus, the majority of monogyne colonies (>80%) were either parasitized with a single organism or were uninfected while the majority of polygyne colonies (>55%) were infected with two or more organisms simultaneously.

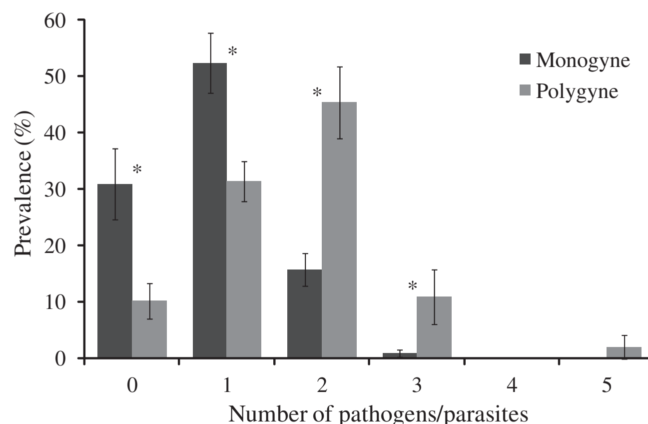

Fig. 2. Prevalence of monogyne and polygyne colonies infected or parasitized with various numbers of pathogens/parasites. An asterisk above the bar pair represents significant ($p < 0.05$) difference between monogyne and polygyne ant colonies by Student's *t*-test. Means were acquired without regard for collection site.

Table 5 summarizes the pathogen/parasite combinations detected in monogyne and polygyne colonies.

SINV-1 and *K. solenopsae* co-occurred 40% ((66–47.2/47.2) ∗ (100)) more often than expected (Table 6). This was due to a larger number of pairs than expected of these two pathogens in monogyne samples at the Paynes Prairie site. This association was not significant with monogyne colonies at the Variable sites (combined)

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Table 5
Summary of pathogen combinations found infecting polygyne and monogyne colonies. Data are presented in descending order based on the total number of colonies in each category.

Pathogens[a]	Number (%) detected in		Total
	Monogyne colonies	Polygyne colonies	
Uninfected	75 (30.9)	12 (10.3)	87 (24.1)
V1	93 (38.3)	25 (21.4)	118 (32.8)
V1K	17 (7.0)	39 (33.3)	56 (15.6)
V3	19 (7.8)	1 (0.9)	20 (5.6)
V1P	8 (3.3)	4 (3.4)	12 (3.3)
V2	7 (2.9)	3 (2.6)	10 (2.8)
K	3 (1.2)	6 (5.1)	9 (2.5)
P	7 (2.9)	1 (0.9)	8 (2.2)
V1V3	5 (2.1)	3 (2.6)	8 (2.2)
V1V2	3 (1.2)	5 (4.3)	8 (2.2)
V1V3K	0 (0)	4 (3.4)	4 (1.1)
V1V2K	0 (0)	4 (3.4)	4 (1.1)
V2P	1 (0.4)	2 (1.7)	3 (0.8)
V1V2V3	0 (0)	3 (2.6)	3 (0.8)
V3K	1 (0.4)	2 (1.7)	3 (0.8)
V1V2P	1 (0.4)	1 (0.9)	2 (0.6)
V3P	1 (0.4)	0 (0)	1 (0.3)
V1KP	1 (0.4)	0 (0)	1 (0.3)
KP	1 (0.4)	0 (0)	1 (0.3)
V2K	0 (0)	1 (0.9)	1 (0.3)
V1V2V3PK	0 (0)	1 (0.9)	1 (0.3)
Σ	***243***	***117***	***360***

[a] Pathogen/parasite key: V1 = SINV-1; V2 = SINV-2; V3 = SINV-3; P = *Pseudacteon* spp.; K = *Kneallhazia solenopsae*.

or the USDA site nor did the pattern hold with polygyne colonies at these same sites. SINV-1 and SINV-3 co-occurred in the same samples about half as often as expected (Table 6). This pattern was found with monogyne samples in all three sites, but not with polygyne colonies. SINV-2 was about twice as likely to co-occur with phorid flies as expected (Table 6) due to samples at the USDA site, but not the Paynes Prairie or the variable sites where the numbers for this cell were low.

4. Discussion

A positive relationship between temperature and SINV-1 prevalence has now been demonstrated in two long-term studies (Valles et al., 2007b). Thus, the relationship is not likely a result of random chance. *Solenopsis invicta* is more active during warmer periods (Porter and Tschinkel, 1987) and colony growth ceases at temperatures below 24 °C (Porter, 1988). Logically, SINV-1 would seem to have a better opportunity to spread more rapidly during warmer periods (Porter, 1988) because the host colonies are actively growing and the frequency with which intra- and inter-colony interactions occur are greater (Porter and Tschinkel, 1987). Indeed, because the virus replicates in the gut epithelial layer and viral particles are shed into the gut lumen (Hashimoto and Valles, 2007), trophallaxis, grooming, and nest maintenance behaviors would normally be expected to facilitate the spread of SINV-1. Virus particles would be transferred to other ants by trophallaxis or contamination of the nest matrix by defecation (Oi and Valles, 2009).

A similar temperature-dependent relationship was observed for the honey bee and some of its associated viruses, and *Drosophila melanogaster* Meigen with a number of single-stranded RNA viruses (Plus et al., 1975; Bailey et al., 1981). With regard to the honey bee, Bailey et al. (1981) hypothesized that viral-contaminated fecal depositions within the hive of infected bees would accumulate during the winter and be rapidly spread throughout the colony during the spring by house-keeping bees. Although increased activity and colony interactions during warmer temperatures is a logical explanation for the corresponding increase in SINV-1 prevalence, it fails to explain why there is a precipitous decline in SINV-1 prevalence during cooler periods of the year. Perhaps environmental conditions (e.g., cooler temperatures) directly affect the ability of SINV-1 to replicate within the ant as opposed to ant behavior influencing SINV-1 spread. In fact, Cevallos and Sarnow (2010) recently reported that Cricket Paralysis Virus (CrPV), also a dicistrovirus like SINV-1, infection resulted in the global inhibition of translation in insect cells and yielded a correspondingly greater production of viral protein and RNA when exposed to elevated temperature (37 °C). However, viral assembly at 37 °C was inhibited. Because insects are poikilotherms, environmental temperature (associated with seasonal changes) could have a direct effect on virus replication and assembly if it is temperature dependent. Or, more simply, cold stress experienced by infected individuals may cause them to die and "fall out" of the population.

Although infection was greatest during the summer and fall, no significant correlation was observed between *K. solenopsae* parasitism and temperature or rainfall. A number of studies have examined this relationship (Briano et al., 1995; Cook, 2002; Milks et al., 2008). Neither Cook (2002) nor Milks et al. (2008) observed a significant correlation between infection and temperature. Milks et al. (2008) and Briano et al. (1995) reported a significant correlation between infection and rainfall. *K. solenopsae* prevalence has generally not exhibited consistent seasonal or climatic trends at various sites in the U.S. or Argentina probably because monitoring encompassed epizootics and/or had varying sampling designs (Briano et al., 1995; Oi and Williams, 2002; Milks et al., 2008). The social form-biased infection by *K. solenopsae* has been observed previously (Oi et al., 2004; Fuxa et al., 2005a; Milks et al., 2008; Oi and Valles, 2009), and although the infection rate of *K. solenopsae* among monogyne colonies varies by study, it is invariably lower in monogyne colonies. Interestingly, the social form bias is not

Table 6
Co-occurrence table of the four pathogens and the decapitating fly parasites in colony samples (both monogyne and polygyne) with observed numbers on top and expected values in parentheses below.

	SINV-1	SINV-2	SINV-3	*Kneallhazia*	*Pseudacteon*
SINV-2	18 (19.4)				
SINV-3	16** (24.2)	4 (3.6)			
K. solenopsae	66** (47.2)	6 (6.9)	8 (8.7)		
Pseudacteon Flies	16 (17.6)	6** (2.6)	2 (3.2)	3 (6.3)	
Total occurrences[a]	218	32	40	78	29
Percent of samples ($n = 360$)	61	9	11	22	8

[a] Total occurrences including samples with only one organism.
** $p < 0.01$; Chi-square tests, 2 by 2 contingency tables.

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observed in the native range for *S. invicta* (Valles and Briano, 2004). The reason for this apparent geographic-dependent disparity among social form infection with *K. solenopsae* is not known; however, it may be based on intercontinental differences in genetic relatedness among polygyne colonies (Ross et al., 1996). South American polygyne colonies typically have fewer queens and greater intra-colonial genetic relatedness than their North American counterparts. The absence of appropriate vectors of this disease may also limit infection of monogyne colonies which are not interconnected like polygyne colonies.

Pseudacteon was likely present in the majority of colonies, but because our sample size of workers was so small ($n = 10$/colony, or about 0.004% of mature colonies comprised of 250,000 workers) flies were only detected in a fraction (8.1%) of the samples. *Pseudacteon* parasitism was more or less uniform throughout the year in Florida. Assuming only one individual worker was parasitized from positive samples, this indicates that at least 0.8% of the individuals from these colonies were parasitized (1/10 of sample individuals times 8.1%). This estimate of parasitism is similar to previously estimated rates (Morrison and Porter, 2005).

Polymorphic social insect species, like *S. invicta*, are thought to be infected with more kinds of parasites than monomorphic species because variably-size workers offer more niches to be exploited by parasites (Schmid-Hempel, 1998). Indeed, *S. invicta* is reported to support a large number (>30) of parasites (Williams et al., 2003). Although both social forms are polymorphic, there was an obvious disparity between monogyne and polygyne colonies with respect to the number of pathogens/parasites infecting a single colony. In the U.S., polygyne colonies have been reported to exhibit low intra-colony genetic relatedness while monogyne colonies exhibit high intra-colony genetic relatedness (Ross et al., 1996). Genetic variability is thought to limit the level of parasite infection within a colony and, consequently, minimize the negative impact of a given parasite from the colony's perspective (Keller, 1995; Schmid-Hempel, 1998). Monogyne colonies exhibit higher genetic relatedness, so these colonies would be expected to have a higher intra-colonial pathogen prevalence when infected (Schmid-Hempel, 1998). However, because pooled groups of worker ants were used in our study, the intra-colony prevalence of pathogens could not be determined.

Polygyne colonies grow by budding (Vargo and Porter, 1989) and the frequent adoption of new queens (Porter, 1991; Tschinkel, 2006). They also frequently exchange workers (Bhatkar et al., 1991) and probably brood. Consequently, infection rates and pathogen diversity among colonies would be expected to be greater in polygyne supercolonies because of the frequent interchange of individuals among colonies. Also, multiple interconnected polygyne colonies would be much more likely to be infected than a single monogyne colony simply because they would represent a larger target. The potentially "immortal" nature of polygyne colonies would also allow diseases to accumulate over time. Conversely, monogyne colonies generally do not exchange workers, brood or queens, and they are also short-lived compared with the interconnected and potentially immortal polygyne supercolonies. Therefore, infections may not persist in monogyne populations (Fuxa et al., 2005a,b).

The poor flight ability, and corresponding decreased dispersal capacity, of polygyne alate reproductives (DeHeer et al., 1999) may also contribute to an increased prevalence of pathogens. Recent pathogen–host population studies have shown that limited dispersal in a population can intensify pathogen effects and lead to higher infection rates (Boots et al., 2009). Therefore, the long-lived nature, the limited flight ability of reproductives (decreased dispersal), and queen adoptions of the polygyne social form likely contribute to an increased prevalence of pathogen infections, as observed.

In addition to showing differences between polygyne and monogyne colonies with respect to the number and species of pathogens/parasites infecting them, our study also revealed that these parasites and pathogens are self-sustaining and present in *S. invicta* all year long (to varying degrees) in Florida. Approximately 60% of all colonies surveyed were infected or parasitized with at least one pathogen or parasite. Even small pathogen effects on colony efficiency and productivity can have profound effects on the competitiveness of a colony (Keller, 1995). Therefore, it is likely that these pathogens and parasites are exerting pressure on *S. invicta* populations in the U.S. Additionally, polygyne colonies may be serving as community reservoirs for these and other pathogens as they were more often infected with multiple organisms, are found interspersed among, and interact with, monogyne populations (Fritz and Vander Meer, 2003), and are long-lived comparatively. This notion is supported by a higher prevalence of *K. solenopsae* in monogyne colonies at the USDA site where polygyne colonies were more prevalent (Table 4).

While *K. solenopsae* in the U.S. is consistently less abundant in monogyne fire ants than in polygyne fire ants (Oi et al., 2004), that does not appear to be the case in Argentina where both forms are apparently infected at similar rates (Valles and Briano, 2004). Several explanations for equal rates of infection in Argentina are possible. The first is that the biotype of *K. solenopsae* in Argentina is somehow different in a way that makes it more infectious to monogyne colonies. A second explanation is that there is a vector for *K. solenopsae* in Argentina that effectively transfers it among both monogyne and polygyne colonies. Phorid flies are known carriers of *K. solenopsae* (Oi et al., 2009), but we do not yet have empirical evidence that they actually vector this pathogen among fire ant colonies. Nevertheless it is of interest that when monogyne and polygyne fire ant colonies were first sampled for *K. solenopsae* in the Gainesville area in 2004, none of the monogyne colonies were infected (Oi et al., 2004). By 2009 when our study was conducted, 9–14% (count or mean %) of monogyne colonies were infected. While one species of *Pseudacteon* fly did occur in Gainesville in 2004, the numbers of flies increased by more than a factor of 10 after the introduction of two additional species (Porter, 2010).

The presence of one organism does not appear to preclude the presence of another. Likewise, we conclude that one organism does not require the presence of another. All combinations of pathogens/parasites (except SINV-2 + SINV-3) were observed (Table 5). Observed values did not differ from random probabilities in 7 of the 10 pairwise combinations (Table 6). SINV-1 was found more often than expected with *K. solenopsae* and less often than expected with SINV-3 (Table 6); however, these relationships were not consistent site to site or between monogyne and polygyne colonies. Similarly, *Pseudacteon* flies were found more often than expected with SINV-2, but again this was not consistent across site or social form and cell numbers were low. Overall, we conclude that these organisms are largely assorted randomly among their hosts; unknown local factors apparently cause occasional deviations from expected values.

Our paper documents that polygyne fire ant colonies are particularly susceptible to pathogen infections, a finding long expected. Susceptibility to infections may be an important reason why polygyne colonies do not displace all or most monogyne *S. invicta* colonies in the U.S. in spite of the advantages of polygyny. It is also of interest to note that the frequency of polygyne colonies in South America is usually lower than it is here in the U.S. (Porter et al., 1992, 1997). Perhaps this situation occurs because there is a greater frequency and variety of pathogens in South America.

Acknowledgments

The authors thank C.A. Strong for technical assistance and Drs. C. Allen and J. Becnel (USDA-ARS) for critically reviewing the

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