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Pseudacteon tricuspis: Its Behavior and Development According to the Social Form of Its Host and the Role of Interference Competition Among Females

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ABSTRACT We studied how the behavior and performance of *Pseudacteon tricuspis* Borgmeier varies with the social form of its host *Solenopsis invicta* Buren, in its native range in Argentina where monogyne colonies are more abundant than polygyne (≈ 75 vs. 25%). Female, *P. tricuspis* took 44% less time (50 vs. 89 s) to attack monogyne than polygyne ants, but oviposition attempts were similar (23 vs. 18 attacks). The presence of the parasitoid affected the average size of foragers on the trail, with the proportion of minor workers increasing on both social forms. In the laboratory, *P. tricuspis* selected similar host sizes, although pupal survival was 25% higher on monogynes than on polygyne. Developmental times of both genders were similar (33–35 d), although larger females emerged from bigger hosts. The sex ratio of *P. tricuspis* was more male biased when exploiting polygyne ants. Intraspecific competition significantly affected parasitoid reproductive success, being significantly higher for a solitary female than when three females were present, although the size of workers selected did not vary. The male:female ratio also changed, being 1:1 without competition but 2:1 with competition. We demonstrated for the first time the consequences of interference competition among *P. tricuspis* females, a common behavior observed in others parasitoids. We discuss why *P. tricuspis* sex ratios are always biased toward males in both social forms and suggest that similar studies of interference competition within and between already naturalized *Pseudacteon* species in the United States could help predict establishment patterns.

KEY WORDS *Pseudacteon tricuspis*, biological control, interference competition, sex ratio, social form

Solenopsis invicta Buren is a pest fire ant species introduced in the United States, probably originating from Argentina (Gilbert and Patrock 2002, Graham et al. 2003, Porter et al. 2004, Thead et al. 2005, Morrison and Porter 2006). The monogyne social form of *S. invicta* is predominant in Argentina and much of the United States, although the polygyne form is dominant from western Louisiana to southern Texas (Greenberg et al. 1985; Porter and Savignano 1990; Porter et al. 1992, 1997; Macom and Porter 1996). The number of mated queens influences the genotype of workers and the colony social form (Keller and Ross 1998, Deheer 2002), which determinates the proportion of subcastes (Chirino et al. 2009). One consequence is that the mean size of *S. invicta* monogyne workers is greater than that of polygyne (Greenberg et al. 1985, Morrison and Gilbert 1998). Furthermore, monogyne colonies are more aggressive toward foreign queens

(Bourke 2002) and workers (Morel et al. 1990) than polygyne.

Several species of phorid flies in the genus *Pseudacteon* Coquillett (Diptera: Phoridae), are used in the United States for biological control. *Pseudacteon tricuspis* Borgmeier was the first to be reared in the laboratory (1997–1999) (Porter et al. 2004, Morrison and Porter 2005a) and several populations are now well established in Florida (Porter et al. 2004, Pereira and Porter 2006) and Texas (Gilbert et al. 2008). This parasitoid is spreading rapidly (LeBrun et al. 2008), and to better understand the potential distribution, dynamics, and impact of introduced *Pseudacteon* species, baseline studies of their performance in native ranges are important.

P. tricuspis, the most abundant and widely distributed species in Argentina and Brazil (Calcaterra et al. 2005, Folgarait et al. 2005a), is a large, sexually dimorphic species with females being larger than males (Morrison et al. 1997, Morrison and Gilbert 1998). Oviposition occurs on larger fire ants of both social forms in the laboratory (Morrison et al. 1997, 1999; Morrison and Gilbert 1998) and in the field (Wood and Tschinkel 1981, Chirino et al. 2009). Because natural percent parasitism is very low (Morrison et al.

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1997, Morrison and Porter 2005a, Calcaterra et al. 2008) the main effect of *P. tricuspis* on *S. invicta* is indirect, modifying the activity pattern and sizes of workers (Morrison and King 2004, Morrison and Porter 2005b).

Understanding a parasitoid's relative preference for each social form could be of importance for biological control efforts. For instance, we found that the social form of *S. invicta* affects development in *P. curvatus* (Chirino et al. 2009), with stronger preference for, and more offspring produced from, monogyne than polygyne fire ant colonies. Consequently, our first goal was to evaluate whether *P. tricuspis* prefers the monogyne over the polygyne form, and to study the parasitoid's effect on the size and proportion of foragers in the field. The second goal was to evaluate life history traits of *P. tricuspis* in relation to the social form of its host in a site where both forms occur in sympatry. Finally, as several *P. tricuspis* females can be found simultaneously attacking a single host colony, we studied the behavior and performance of a single female versus several conspecific females under laboratory conditions.

Materials and Methods

Ant and Fly Sources. Experiments were carried out between November 2006 and October 2007 near San Javier City (30° 35'S and 59° 55'W), Santa Fe Province, Argentina. This site is a subtropical forest with an average precipitation of 1,170 mm a year (Cabrera and Willink 1980). Ants occurring in pastures around the forest were identified using keys by Trager (1991), Pitts (2002), and Pitts et al. (2005). The social form (Monogyny: one queen, Polygyny: more one queen) of each *S. invicta* colony was determined by collecting one mound and counting the number of queens. Clumped mounds separated by <1 m were considered the same polydomic nest; only the largest mound was excavated. Polydomic colonies are mainly observed in the polygynic social form of *S. invicta* because the queens frequently mate in the nest and disperse only short distances from their natal nest (Tschinkel 1998, Goodisman et al. 2000). The field-collected queens were mated as they produced workers when set up in the laboratory. Colonies with 0 or 1 queen were considered Monogyny if the specific DNA segment of the *Gp-9^B* gene was present (Valles and Porter 2003; M.G.C., unpublished data). Three measurements were taken from six mounds of each social form for all observations in the field and the laboratory. *P. tricuspis* females collected in the field were identified with a 20× magnifying lens (Porter and Pesquero 2001), and used within 48 h after collection. All measurements of ant and phorid head capsule widths were taken using an ocular micrometer, calibrated in 0.03 mm increments, in a stereoscopic microscope (Nikon Japan model SMZ-1B ESD, Microlab S. R. L., Buenos Aires, Argentina). Voucher specimens of *P. tricuspis* and *S. invicta* are deposited at Centro de Estudios e Investigaciones, Universidad Nacional de Quilmes and

Museo de Historia Natural Bernardino Rivadavia (Buenos Aires, Argentina).

Field Data. We assessed ant foraging activity in absence of the parasitoid, as well as the sizes of workers and the proportion of foragers before exposure and after the test fly had been removed. Tuna baits were placed 20 cm from each mound and once a foraging trail was established, the colony was isolated with acrylic transparent tunnels (1 × 0.15 × 0.20 m) to exclude other parasitoids or rival ants. Forty-five minutes after a trail was established we determined the number foragers moving past a fixed point during a 5 min period, and collected a random sample of 50–150 ants. We then released a *P. tricuspis* female into the tunnel for 5 min and measured 1) the reaction time (time invested in detecting ants before beginning attacks), and 2) the number of attacks ("ovipositions"). The fly was recaptured and a second random sample (50–150 individuals) of worker ants on foraging trail/bait was collected. This allowed us to estimate the proportion of different sized foragers (based on head capsule width) on the trail before and after the parasitoid was present. Each parasitoid was used once. Because data were normally distributed, reaction times and number of attacks of *P. tricuspis*, as a function of the social form of the ant colony, were analyzed using Student's *t*-tests. Relationships between number of attacks and reaction time, as well as number of attacks as a function of the number of foragers, were analyzed using linear regressions.

After exposure to the parasitoid the mound was excavated, the number of queens counted and workers then sorted into the following five size categories using different sized ZONYTEST sieves (Rey & Ronzoni S. R. L., Buenos Aires, Argentina): major (ants retained in the sieve mesh #16, with a pore size of 1,190 μm); large (retained in the sieve mesh #18, with a pore size of 1,000 μm); medium (retained in the sieve mesh #20, with a pore size of 840 μm); small (retained in the sieve mesh #25, with a pore size of 710 μm); and minor (passed through the sieve mesh #25, with a pore size of 710 μm).

The number of active foragers in the 5 min observational period were normally distributed so differences between social forms were compared using the Student's *t*-test. Mean forager size before and after releasing phorids were analyzed with paired *t*-tests, and the mean sizes of subcastes within each social form compared using one-way analysis of variance (ANOVA). The proportion of different sized workers foraging and defending the bait before, and after releasing phorids, were compared by means of the proportion test.

Laboratory Rearing. Evaluation of Life History Traits of Parasitoid Adults. An arena was set up with 667 large, medium, and small workers from either a monogyne or a polygyne colony, and three wild *P. tricuspis* females were introduced for 2 h. After this exposure the ants were held at 28 ± 1°C, 80 ± 10% RH and cadavers were collected daily. The head capsules of parasitized cadavers were measured (width across the eyes) and kept for 60 d so that pupal survival, as

Table 1. Average size (mean \pm SE; mm) and proportion of different *S. invicta* workers on the trail before and after a *P. tricuspis* female was introduced, as a function of the social form of the ant nest

Social form	Workers size and proportion by subcaste ^b					F ^c	P
	Major	Large	Medium	Small	Minor		
Monogyny							
Size	1.06 \pm 0.02	0.95 \pm 0.03	0.80 \pm 0.02	0.65 \pm 0.02	0.53 \pm 0.01	95.90	< 0.0001
Before	0.017	0.040	0.077	0.271	0.597		
After	0.020	0.034	0.045	0.186	0.715		
Change ^a	-	-	↓	↓	↑		
Polygyny							
Size	1.03 \pm 0.01	0.94 \pm 0.01	0.73 \pm 0.02	0.62 \pm 0.01	0.52 \pm 0.005	373.85	< 0.0001
Before	0.014	0.027	0.122	0.283	0.554		
After	0.017	0.022	0.116	0.249	0.596		
Change ^a	-	-	-	↓	↑		

^a Changes in the proportions for each subcaste, in absence and presence of *P. tricuspis*, were analyzed by the Proportion Test Arrows (\uparrow , \downarrow) indicate the direction for significant changes ($P < 0.05$).

^b Comparison for each worker subcaste between monogyny and polygyny was analyzed with Student's *t*-test. Only medium workers differed statistically ($t = 2.41$, $df = 10$, $P = 0.0367$; others $P > 0.05$).

^c One-way ANOVA comparisons between mean sizes of subcastes. All subcastes differed statistically ($P < 0.05$).

well as sex and size of emerging adult parasitoids (maximum width of pronotum), could be assessed.

The sizes of parasitized ants in the two social forms were compared with the Student's *t*-test, while those of females, males, and inviable parasitoid pupae (width of parasitized head capsule) were compared using a one-way ANOVA with Bonferroni adjusted a posteriori contrasts. To evaluate the relationship between social form and sex of the adult parasitoids, we ran two-factor analyses of covariance (analysis of covariance [ANCOVA]) on thoracic widths of flies, with head width of ants as the covariate. Sizes of parasitoids and hosts were compared by linear regression. To control for type 1 error ($\alpha = 0.05$) in multiple comparisons made on the size of host selected and the size of the resulting phorid a posteriori contrasts were performed with adjusted significant levels using the sequential Bonferroni procedure ($P < 0.007$; Sokal and Rohlf 1969). Differences in the sex ratio of emerging parasitoids were determined by the binomial tests, while pupal survival in the different treatments (the proportion pupae giving rise to viable adults) were analyzed with proportions tests.

Evaluation of the Performance of a Single Female Without Competition. We examined whether selected worker sizes, number of ovipositions, reproductive success, and sex ratios of parasitoids were affected in the absence of competition for the resource as ant densities changed over time. One laboratory reared *P. tricuspis* female (mated with one male) was placed with 667 large, medium, and small monogyny ants (2,000 total) in each trial ($N = 7$). Each female was allowed remain in the arena until losing motivation (no more attempted ovipositions) that generally occurred before 3–5 h. All measurements of parasitoid performance were conducted over 10 min intervals. Each ant that was attacked ("touched" in the pleura near the legs) was collected using a manual aspirator, its size measured, and then reared together with the other individuals attacked during the same time interval.

The number of oviposition attempts, the number of successful attacks (the number of ovipositions that

resulted in pupae) and the size of hosts selected in the different 10 min periods were analyzed using one-way ANOVA and the sequential Bonferroni correction method for multiple comparisons. The average size of parasitized ants and resulting parasitoid adults were compared by the Student's *t*-test. Pupal survival in different treatments was analyzed with the proportions test, while the sex ratio of adults was determined by a binomial test. Statistical analyses were done using Statview software (SAS Institute Inc. 1992–1998).

Results

Field Data. Effects of *P. tricuspis* on *S. invicta* Foragers. In the absence of *P. tricuspis*, the number of individuals foraging was significantly lower for monogyny than polygyny colonies (500.5 ± 43.6 and 539.3 ± 43.0 ants; $t = 1.71$, $df = 10$, $P = 0.04$) although the mean head width was slightly larger for monogyny than polygyny workers (0.62 ± 0.01 and 0.59 ± 0.01 mm for monogyny and polygyny workers, respectively; $t = 1.89$, $df = 10$, $P = 0.04$). Regardless of the social form of the ant nest, once a *P. tricuspis* female was introduced, ants stopped foraging and left the bait to hide or exhibit stereotypical freezing-postures. However, once foraging was resumed, after the parasitoid was removed, the size of foraging monogyny workers had diminished significantly ($t = 2.97$; $df = 10$; $P = 0.016$) but it remained unchanged for polygyny workers ($t = 1.69$; $df = 10$; $P = 0.07$) and so the size of both monogyny and polygyny frozen foragers ants were similar (0.57 ± 0.01 and 0.58 ± 0.01 mm; $t = 0.66$, $df = 10$, $P = 0.52$).

The presence of *P. tricuspis* affected the proportion of worker subcastes foraging in both monogyny and polygyny colonies, as there were significant shifts in the relative proportions of worker classes before and after the parasitoid was introduced (Table 1).

Preferences of *P. tricuspis*. *P. tricuspis* took significantly less time to begin attacking foragers from monogyny than polygyny colonies (50.3 ± 11.4 vs. 89.2 ± 42.2 s; $t = 4.56$, $df = 10$, $P < 0.001$), but the number of attempted ovipositions during the test periods were

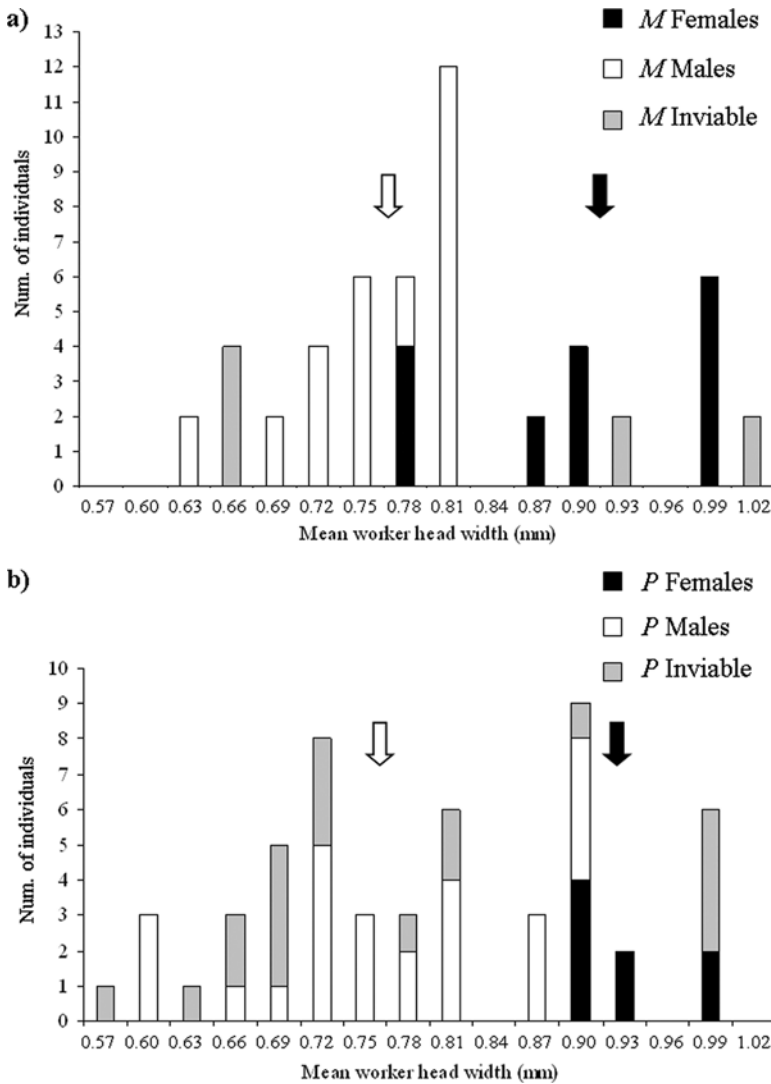


Fig. 1. Head sizes of *S. invicta* workers selected by *P. tricuspis* females when exploiting (a) monogyne (*M*) or (b) polygyne social form (*P*) of the host. Arrows indicate the mean size for those producing female (black) and male (white) parasitoids.

similar (23.2 ± 5.1 and 17.7 ± 3.0 attacks; $t = 0.94$, $df = 10$, $P = 0.816$). No significant relationship was found between the number of attacks carried out by *P. tricuspis* and the number of foragers present on the trails ($R^2 = 0.02$, $P > 0.90$ and $R^2 = 0.18$, $P = 0.40$ for monogyne and polygyne ants, respectively). There was a negative relationship between the reaction time and the number of attacks when *P. tricuspis* was in the presence of a monogyne colony ($R^2 = -0.756$; $P = 0.0245$) but not with a polygyne one ($R^2 = -0.154$; $P = 0.44$).

Laboratory Rearing

Evaluation of Life History Traits of Parasitoid Adults. Worker Castes Selected. *P. tricuspis* oviposited on similar sizes of workers regardless of the social form

of the *S. invicta* colony (0.79 ± 0.03 and 0.82 ± 0.02 mm for monogyne and polygyne nests, respectively; $t = 0.67$, $df = 7$, $P = 0.53$). Female progeny emerged from larger hosts than males for both monogyne (0.92 ± 0.03 vs. 0.76 ± 0.02 mm; $t = 4.62$, $df = 7$, $P = 0.0024$) and polygyne (0.93 ± 0.01 vs. 0.77 ± 0.03 mm; $t = 4.18$, $df = 6$, $P = 0.0058$) colonies. In monogyne colonies, 66.7% of females emerged from large and 33.3% from medium workers, whereas 86.7% of the males emerged from medium and 13.3% from small workers. In polygyne colonies, females only emerged from large workers, while 26.9% of the males emerged from large, 53.8% emerged from medium and 19.2% from small workers (Fig. 1).

In monogyne colonies the inviable pupae were only found in the heads of the smallest and largest hosts exploited, whereas in polygyne colonies they oc-

Table 2. *P. tricusps* output in the presence of competing conspecific females (on both social forms of *S. invicta*) and without female competition for monogynic colonies

Productivity	With-competition		Without-competition
	Monogyny	Polygyny	Monogyny
No. trials	6	6	7
No. females per trial	3	3	1
No. total pupae	54	53	66
No. total adults	46	34	50
Total females	16	8	26
Total males	30	26	24
Ratio female:male	0.53	0.31	1.08
Pupal survival (%)	85.2	64.2	75.8
Efficiencies on the development			
No. pupae/female	3	2.9	9.4
No. adults/female	2.6	1.9	7.1

curred over the whole range of host sizes attacked (Fig. 1). Parasitoid pupae were obtained in 83% of all trials, and the average number was similar in both social forms (13.5 and 13.3 pupae for monogynic and polygynic nests, respectively; $Z = 0.27$, $P = 0.50$). However, pupal survival was significantly higher in ants from monogynic than polygynic nests (85.2 vs. 64.2%; $Z = 2.28$, $P = 0.02$) (Table 2).

Developmental Periods. Larval, pupal and total developmental times of *P. tricusps* did not vary between sexes nor were they affected by the social form of the host (Table 3).

Characteristics of Adult Flies. There was a sexual size dimorphism in *P. tricusps* with females being larger ($F = 126.55$; $df = 1, 71$; $P < 0.0001$) that was associated with the size (width) of the host used ($F = 31.30$; $df = 1, 71$; $P < 0.0001$), but not the social form of *S. invicta* ($F = 0.04$; $df = 1, 71$; $P = 0.84$). There was also a positive relationship between the size of the host and the size of the resulting parasitoid from both monogynic (Fig. 2a) and polygynic (Fig. 2b) colonies, and although females came from bigger hosts the slopes were not significantly different between genders ($P > 0.05$ in both social forms). Sex ratio was biased toward males and varied between social forms; females represented 35% ($P = 0.027$) and 24% ($P = 0.00147$) of adults for monogynic and polygynic colonies, respectively (Table 2).

Performance of a Single Female Without Competition. *Ovipositions and the Incidence of Parasitism.* The mean time a solitary *P. tricusps* female spent ovipositing was 37.1 ± 7.2 min. While the number of attacks declined with time ($F = 5.58$; $df = 4, 21$; $P = 0.0032$), the mean size of workers selected did not vary (0.88 ± 0.01 mm; $F = 2.49$; $df = 6, 19$; $P = 0.06$; Table 4). All females attacked a similar number of hosts ($F = 2.12$; $df = 6, 19$; $P = 0.0984$), but the proportion of attacks that yielded parasitoid pupae only averaged 5.8% for all females tested (Table 4). Thus, the number of attacks observed is not a good indicator of fitness, given that no significant relationship was observed between this variable and the number of pupae produced ($R^2 = 0.183$, $P = 0.3386$). As in the other experiments, female parasitoids were larger than males (0.50 ± 0.01 vs. 0.39 ± 0.01 mm; $t = 8.59$, $df = 43$, $P < 0.0001$) and emerged from larger hosts (1.01 ± 0.01 vs. 0.82 ± 0.02 mm; $t = 6.87$, $df = 43$, $P < 0.0001$). However, while pupal survival was very similar in the presence or absence of competition, the sex ratio was 1:1 rather than being male biased when there were competing parasitoid females (Table 2).

Both female (0.50 ± 0.01 vs. 0.46 ± 0.01 mm for females; $F = 7.59$; $df = 2, 45$; $P = 0.0014$) and male (0.39 ± 0.01 vs. 0.36 ± 0.01 mm for; $F = 3.73$; $df = 2, 72$; $P = 0.028$) parasitoid progeny were bigger in the no competition than in the competition assays (Table 2). Females emerged from larger ants in the no-competition than in the competition treatment (1.01 ± 0.01 vs. 0.92 ± 0.03 mm; $F = 11.07$; $df = 2, 45$; $P = 0.0001$) but this was not the case for males (0.82 ± 0.02 vs. 0.76 ± 0.02 mm; $F = 2.33$; $df = 2, 72$; $P = 0.1021$). Overall, the performance of a parasitoid female was three times higher in a no-competition than a competition situation (Table 2).

Discussion

This study demonstrated that *P. tricusps* has a significant effect on the behavior and foraging pattern in the monogynic social form of *S. invicta*. In the laboratory, *P. tricusps* successfully developed on both social forms, but its reproductive success was 24.7% higher on monogynic colonies (Table 2). We also showed, for the first time, the negative effect of female intraspe-

Table 3. Average developmental times (d) and size (mean \pm SE; mm) of *P. tricusps* females and males as a function of the social form of their fire ant host

Social form	Sex	N (n) ^a	Developmental periods (d)				Phorid size (mm)	P ^b		
			Larval	P	Pupal	P			Total	P
Monogyny	Females	4 (16)	17.8 \pm 1.4	0.20	16.6 \pm 0.2	0.40	34.3 \pm 1.5	0.36	0.5 \pm 0.02	0.0007
	Males	5 (28)	15.7 \pm 0.6		17 \pm 0.5		32.8 \pm 0.7		0.4 \pm 0.01	
Polygyny	Females	3 (8)	18.8 \pm 0.8	0.33	16.5 \pm 0.01	0.77	35.3 \pm 0.8	0.16	0.5 \pm 0.01	0.0005
	Males	5 (26)	17.2 \pm 1.1		16 \pm 0.4		33.1 \pm 0.9		0.4 \pm 0.01	
Between ^c	Females			0.58		0.38		0.61		0.75
	Males			0.29		0.12		0.78		0.52

^a N indicates the no. of nests from which females and males emerged, and n the total no. of parasitoids that emerged.

^b Comparisons between sexes within social forms were analyzed with the two-tailed Student's *t*-test.

^c Comparisons for each sex between social forms were analyzed with the two-tailed Student's *t*-test.

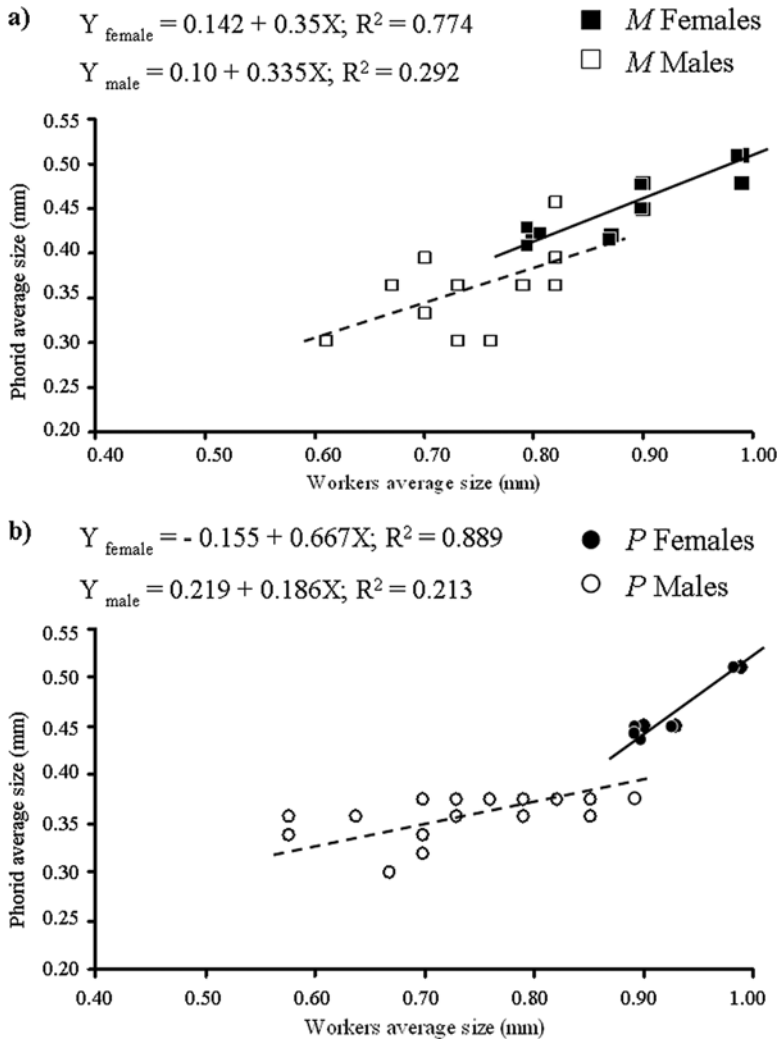


Fig. 2. Linear regression between *S. invicta* host worker size and females and males of *P. tricuspis* exploiting either the (a) monogyne (*M*) or (b) polygyne social form (*P*) of the host. Females are indicated by filled symbols and males by empty ones.

cific competition, as evidenced by a change in sex ratio favoring males (41.5%) and a lower efficiency in production of progeny (35.7%).

In the field, *P. tricuspis* affected the proportion of different monogyne workers, as evidenced by a decrease in the number of medium and small foragers on the trail, and an increase in minor workers, thereby modifying the availability of preferred host sizes (Table 1). These changes may be the result of selection, because monogyne colonies are 2.27 times more abundant than polygyne colonies at our field site (M.G.C., unpublished data). *P. tricuspis* parasitoids also affected the behavior of *S. invicta* workers as larger ants retreated to the nests holes, whereas smaller ants stayed on or near the baits exhibiting frozen postures.

Monogyne colonies have larger but fewer foragers than polygyne ones, a reverse trade-off pattern previously reported for *S. invicta* in Chaco (Chirino et al.

2009). Trade-offs between offspring size and number are common in many animals (Fox and Czesak 2000) and social insects are no exception (Hölldobler and Wilson 1990).

While female parasitoids emerge from larger hosts than males, the size of both sexes is affected by host size (Fig. 2) the sex ratio was more biased toward males when polygyne colonies were exploited. The reasons for this difference could be 1) that polygynic ants are generally smaller than monogynes (0.73 ± 0.01 vs. 0.80 ± 0.01 mm for polygyne and monogyne workers, respectively; $P < 0.001$; M.G.C., unpublished data; Greenberg et al. 1985, Morrison and Gilbert 1998), and/or 2) that polygyne nests have twice as many medium but half the number of large workers than monogyne nests (Table 1). Although, *P. tricuspis* successfully developed in both social forms, the percentage of inviable pupae was higher in polygyne

Table 4. Number of attacked ants, avg. size (mm), no. of attacks, no. of developed pupae and percentage of parasitism (%) of selected workers by a single *P. tricuspsis* female attacking *S. invicta* colonies, discriminated by trial and time intervals (min)

Trial	Interval (min)	No. attacked ants	Average size (mm)	F^a	P	Average no. of attacks	No. pupae	Percentage of parasitized (%)
1	0-10	62	0.94 ± 0.02	2.52	0.08	38.83 ± 6.12	11	4.70
	10	52	0.89 ± 0.02					
	20	33	0.90 ± 0.03					
	30	33	0.87 ± 0.03					
	40	31	0.88 ± 0.03					
2	0-10	59	0.85 ± 0.02	1.58	0.19	44.75 ± 6.15	5	2.79
	10	50	0.88 ± 0.02					
	20	39	0.90 ± 0.02					
	30	31	0.91 ± 0.02					
	40	31	0.88 ± 0.02					
3	0-10	50	0.88 ± 0.02	0.16	0.85	38.00 ± 9.61	5	4.42
	10	45	0.86 ± 0.02					
	20	19	0.86 ± 0.04					
	30	19	0.86 ± 0.04					
4	0-10	80	0.88 ± 0.02	1.11	0.33	60.63 ± 12.20	6	3.32
	10	63	0.91 ± 0.02					
	20	38	0.91 ± 0.02					
5	0-10	50	0.88 ± 0.03	0.52	0.72	34.60 ± 5.11	31	17.71
	10	30	0.88 ± 0.04					
	20	39	0.84 ± 0.03					
	30	35	0.85 ± 0.03					
	40	19	0.91 ± 0.04					
6 ^b	0-10	55	0.89 ± 0.03			55	0	0.00
7	0-10	33	0.87 ± 0.02	0.25	0.86	26.75 ± 2.50	8	7.55
	10	27	0.86 ± 0.03					
	20	28	0.84 ± 0.03					
	30	20	0.84 ± 0.03					

^a Size comparisons across time for each trial were analyzed by means of one-way ANOVA ($P < 0.05$).

^b Female in trial 6 attacked only for the first 10 min.

colonies, which are far less abundant than monogyne ones in Argentina (Wojcik 1983, Ross and Trager 1990, Porter et al. 1997, Ross et al. 1997, Mescher et al. 2003, Chirino et al. 2009). Thus *P. tricuspsis* may be poorly adapted to the polygynic form of *S. invicta* which is 2–3 times more abundant than monogyne colonies in some places in the United States (Macom and Porter 1996).

The results of our laboratory assays suggest that the male biased sex ratio observed (Morrison and Gilbert 1998, Morrison and Porter 2005a) may be partly because of intraspecific competition between foraging *P. tricuspsis* females (Table 2). This results from a lower number of hosts than can support the development of female parasitoids and a smaller number of adults (Table 2). Furthermore, although *S. invicta* colonies may appear to be similar, their quality as hosts may differ because both the incidence of parasitism and pupal viability varied between female parasitoids, even when the number of attacks was similar. Alternatively, the differences observed could be because of interfemale variations regarding their fitness as parasitoids.

Interference competition among *P. tricuspsis* females is common, with direct contact occurring between conspecifics attempting to oviposit. This interference competition pattern was not observed in *P. curvatus*, another species already released in the United States along with *P. tricuspsis* (Porter and Gilbert 2004, Gilbert et al. 2008). The aggressive and territorial behavior observed for *P. tricuspsis* females may be unnecessary as *P. curvatus* mainly attack small workers, which are abundant, and both genders develop in similar host

sizes (Chirino et al. 2009). Moreover, the number of pupae and adults produced by *P. curvatus* under similar rearing conditions were four times higher than *P. tricuspsis* (Vazquez et al. 2004, Chirino et al. 2009). While the overall host size selected by *P. curvatus* is smaller than that chosen by *P. tricuspsis*, *P. curvatus* exploits those workers that *P. tricuspsis* requires to produce males. Thus, when in sympatry *P. tricuspsis* has a strong female-biased operational sex ratio and is competitively displaced (LeBrun et al. 2009).

It is evident from previously published work and the results of this study that one needs to determine the impact parasitoids have on the relative proportion of foragers (Folgarait and Gilbert 1999, Chirino et al. 2009, this study), as well as the behavior and foraging rate of workers (Morrison 1999, Wuellner et al. 2002, Mottern et al. 2004, Chirino et al. 2009, this study) when considering their efficacy as biological control agents. Furthermore, greater attention needs to be given to understanding the effects of both intra and interspecific competition between introduced parasitoid species.

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