

## Development of *Pseudacteon obtusus* (Diptera: Phoridae) on *Solenopsis invicta* and *Solenopsis richteri* Fire Ants (Hymenoptera: Formicidae)

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**ABSTRACT** We studied the developmental performance of the large biotype of *Pseudacteon obtusus* Borgmeier, a promising biological control agent of imported fire ants. We measured selected life history traits of this parasitoid as a function of (1) host species (*Solenopsis invicta* Buren versus *Solenopsis richteri* Forel), (2) temperature (22 versus 28°C), and (3) size distributions of available host ants (big versus mixed-size classes of workers). Survivorship on *S. invicta* was much greater than that on *S. richteri* under most conditions. Developmental time was strongly influenced by temperature with total developmental periods shortened by 21–34% at the 6°C higher temperature treatment. However, developmental periods were weakly influenced by hosts across temperatures. We found that larval, pupal, and total developmental periods of this phorid fly were up to 6.2% longer on *S. richteri* than on *S. invicta*, although these periods depended on temperature. Total developmental time was slightly shorter (by 4.8%) on *S. invicta* than *S. richteri* at 22°C but longer (by 5.7%) on *S. invicta* at 28°C. The relationship between host size and sex of emerging flies contrasts with that of previously documented *Pseudacteon* species, in that males were produced from all host size classes while females only came from larger ants. Sex ratios favor females when a mixture of ant size classes were offered in comparison to when only big ants could be chosen. *Pseudacteon obtusus* seems to be a promising candidate for biological control releases in that in addition to its high host specificity, it develops better on red fire ants, attacks ants on trails and mounds, and chooses a worker size range that complements the two other *Pseudacteon* species already released.

**KEY WORDS** Argentina, biological control, developmental times, parasitoid, fire ants

THE DECAPITATING FLIES OF the genus *Pseudacteon* have received considerable attention recently because of their use in classical biological control against the imported fire ants, *Solenopsis invicta* Buren and *S. richteri* Forel, in the southern United States (Gilbert and Patrock 2002, Graham et al. 2003, Porter et al. 2003). Important reasons given for advocating their application as classical biological control agents against these ants include their highly specific use of hosts within the fire ant complex of *Solenopsis* (Porter et al. 1995a, Gilbert and Morrison 1997, Porter and Alonso 1998, Morrison and Gilbert 1999, Folgarait et al. 2002a), their negative impact on fire ant food retrieval (Feener and Brown 1992, Orr et al. 1995, Folgarait and Gilbert 1999), and their considerable interspecific variation in behavior and distribution patterns in parallel with fire ants in South America (Wuellner et al. 2002, Folgarait et al. 2003, 2005).

The interaction between parasitoid phorid flies and their hosts begins when the flies are attracted to ants foraging for food or performing other outside duties. Disturbed by the presence of the flies, ants will strike up defensive postures (Wuellner et al. 2002) and discontinue normal activities (Folgarait and Gilbert 1999). The female fly will find an appropriate ant and inject an egg in mid-flight into the thorax of the worker. After hatching, the larva begins feeding in the alitrunk and later moves into the head of the ant, where at some point, its feeding activities kill the ant (Porter et al. 1995b, Consoli et al. 2001) and causes the head to separate from the ant's body. Pupariation occurs within the severed head capsule, and adult emergence follows several weeks later.

One of our primary assumptions about the classical biological control of fire ants using *Pseudacteon* flies is that establishment of a set of fly species will be preferable to a single species and that such a set may be necessary to affect observable population regulation. *Pseudacteon* communities in South America consist of 5–10 species (Orr et al. 1997, Folgarait et al. 2003) that

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partition their consumption of the ants along a number of environmental axes (Morrison 2000), including ant size (Campiolo et al. 1994), time of day (Pesquero et al. 1996), seasonal occurrence (Fowler et al. 1995, Folgarait et al. 2003), and ant activity, density, or location (Orr et al. 1997). The introduction of complementary species would expand the breadth and magnitude of their impact on fire ant populations.

Our laboratory has focused its attention on establishment and release of *Pseudacteon* in Texas, where climatic conditions are some of the hottest and driest within the range of the imported fire ants (Gilbert and Patrock 2002). The first step toward meeting our long-range goals is to identify species able to survive under a wide range of variable conditions, but especially those characteristic of Texas. In this regard, *Pseudacteon obtusus* Borgmeier is a promising candidate.

This fly has one of the broadest distributional ranges of the 20 *Pseudacteon* species associated with *Solenopsis* in South America (Folgarait et al. 2005). Its range extends from subtropical to temperate habitats varying from savanna to forests (Folgarait et al. 2005). Overlapping the fly's range is the complex mosaic of its host species' distributions, including *S. invicta* and *S. richteri* (Trager 1991). The wide climatic variation (Folgarait et al. 2005) encompassing the distribution of this species suggests that this species or biotypes of this species have the inherent physiological plasticity to deal with the variable climatic conditions found in Texas. Folgarait et al. (2002a) found that *P. obtusus* from two Eastern Argentinean populations performed better on *S. invicta* than *S. richteri*, suggesting some degree of host specialization for this species, as found by Porter and Briano (2000) for *P. curvatus* Borgmeier.

A potential problem in matching *Pseudacteon* species with *Solenopsis* fire ants in Texas has been host size (Gilbert and Patrock 2002). In two of three *Pseudacteon* species, sex determination is dependent on host size (Morrison et al. 1999, Folgarait et al. 2002b). Polygyne, or multiple queen colonies, are the more prevalent social form in Texas (Porter et al. 1991). Polygyne fire ants produce mostly small workers, and male *Pseudacteon* are produced from smaller ants; therefore, the resulting fly sex ratios are skewed toward males, which are smaller than the females (Morrison et al. 1999). The relationship between host size and sex ratios is therefore an important consideration in predicting the future reproductive performance of these flies on *S. invicta*. We studied inter-relationships between size and development time for *P. obtusus*, because adult size is dependent on host size in other *Pseudacteon* species, and fecundity and other aspects of life history performance are often influenced by body size (Calder 1984, Atkinson 1994). This study evaluates the effects of temperature and host species and host size on development and performance of *P. obtusus*.

### Materials and Methods

Details of our methods and rearing conditions are discussed in greater depth and follow those in Folga-

rait et al. (2002b). Here we briefly overview our methods and provide specific information that differs from that study.

Adult *P. obtusus* (large biotype) used in this study were collected from various localities around Mercedes, Corrientes, Argentina, (29°47' S and 58°03' W). Specimens were collected in the field two to three times per week from September 2000 to November 2001; voucher specimens are located in Folgarait's collection at the Universidad Nacional de Quilmes. Collected flies were placed in separate plastic vials (10 by 40 mm) and transported in a cooler at ≈10°C to our laboratory in Buenos Aires. Flies were tested no >48 h after collection in the field. Ants used in this study came from healthy, monogyne colonies kept for <6 mo in the laboratory. *S. invicta* colonies were obtained from the same general area around Mercedes, Corrientes, as were their parasitoid flies, whereas *S. richteri* colonies derived from the Reserva Ecológica Costanera Sur (RECS), Buenos Aires (34°37' S and 58°22' W).

Typically, we ran two paired oviposition trials per day: one with *S. invicta* ants and the other with *S. richteri*. Two to six female flies were released into flight boxes containing 0.8 g of either species. The flies were allowed to attack for 3–5 h or until they stopped ovipositing or died. To determine whether flies elected to oviposit on a particular size class, we offered them one of two size choices: big and mixed. Big ants were those that failed to pass through soil sieve size 18. The mix choice was represented by 0.20 g of big, 0.22 g of medium (collected on sieve 20), and 0.38 g of small ants (passed through sieve 20).

After their exposure to the flies, the ants were assigned to one of two temperature regimens: 22 and 28°C. All attacked ants were inspected daily for 45 d for the appearance of pupae in the heads of dead ants. Likewise, pupae in their host's head capsule were inspected daily until adult fly emergence. Egg-larval developmental times (days) were calculated as time elapsed since the day of the attack to the day we observed the pupae. Pupal development time (days) was calculated as the time from appearance of a pupa to adult eclosion.

Survivorship was measured directly at the pupal stage. Egg and larvae survivorship was not determined because they could only be observed with destructive sampling because *Pseudacteon* are endoparasitoids. The replicate used to measure survivorship was the cohort of pupae arising from each oviposition trial. We also recorded two proxy variables to estimate lifetime survivorship schedules. Adult efficiency was the number of adult progeny per oviposition trial divided by number of females used in that oviposition trial. We also measured attack efficiency as the percentage of oviposition trials that gave some pupae. These measurements included oviposition rates, as well as egg-larval survivorship, whereas adult efficiency also incorporated pupal survivorship.

Head width of ants offered (big and mixed) versus that of parasitized ant heads was measured to determine if phorid elected to oviposit on a certain ant size

Table 1. Developmental times of *P. obtusus* on *S. invicta* and *S. richteri* at 22 and 28°C

Host	Temperature (°C)	Sex	N	Developmental time ± SE (d)		
				Larval	Pupal	Total
<i>S. invicta</i>	22	Female	125	21.7 ± 0.66	27.0 ± 0.32	48.7 ± 0.77
		Male	215	21.9 ± 0.43	25.1 ± 0.29	47.0 ± 0.50
	28	Female	33	19.0 ± 1.15	19.3 ± 0.70	38.3 ± 1.45
		Male	81	16.4 ± 0.54	18.8 ± 0.63	35.2 ± 0.87
<i>S. richteri</i>	22	Female	21	20.7 ± 1.44	28.8 ± 0.86	49.5 ± 1.75
		Male	55	22.4 ± 0.82	27.7 ± 0.58	50.1 ± 1.07
	28	Female	10	14.6 ± 0.76	18.1 ± 0.67	32.7 ± 0.53
		Male	15	16.6 ± 1.08	18.9 ± 1.05	35.5 ± 1.80

class and to assess how ant size influenced sex ratios. Phorid body size was measured using maximum thoracic width. Measurements were made using an ocular micrometer calibrated in 0.03-mm increments on a microscope (model type 102; Nikon, Japan).

We completed three-factor analysis of variances (ANOVAs; host species, temperature, and sex) when square-root transformed data showed normality and equal variances. Pupal developmental times did not meet these assumptions, so we rank-transformed the values for this variable before conducting the ANOVA. All multiple comparisons were adjusted using Bonferroni's method to keep the  $\alpha = 0.05$  (Daniel 1990). Kolmogorov-Smirnov and Mann-Whitney non-parametric tests were used to compare head size distributions and medians, respectively. These are reported as  $\chi^2$  and Z normal approximations, respectively. To evaluate the relationships between developmental times and ant and fly sizes, we ran a stepwise regression of thoracic widths against their respective developmental times and host ant head widths. Statistical analyses were performed using Statistix (Analytic Software 1998, Tallahassee, FL) and SAS (V.8 1990; SAS, Cary, NC).

## Results

**Host Species Range.** *Pseudacteon obtusus* females originating from a population using *S. invicta* (natural host) readily attacked *S. richteri* workers (novel host of the same species complex). Pupae per female was significantly lower on *S. richteri* (median = 0.78,  $N = 48$ ) than on *S. invicta* (median = 1.0,  $N = 92$ ;  $Z = 2.29$ ,  $df = 1$ ,  $P = 0.02$ ). Accordingly, the efficiency of adult production (adult offspring/female parent) was 150% higher on *S. invicta* than *S. richteri* ( $Z = 3.29$ ,  $df = 1$ ,  $P = 0.001$ ). This difference was because in part of the significantly lower pupal survivorship in *S. richteri* (46.4% lower than in *S. invicta*;  $Z = 2.75$ ,  $df = 1$ ,  $P = 0.006$ ). Likewise, the percentage of oviposition trials that gave place to pupae was greater for *S. invicta* than for *S. richteri* (75 versus 63%).

Developmental time did not differ significantly between hosts for either stadia ( $F_{1,547} = 1.23$ ,  $P = 0.27$  and  $F_{1,547} = 1.98$ ,  $P = 0.16$  for larval and pupal times, respectively) or for total developmental time ( $F_{1,547} = 0.87$ ,  $P = 0.35$ ). We also did not find a significantly different larval developmental time between viable and nonviable pupae ( $F_{1,809} = 0.22$ ,  $P = 0.64$ ). As a

whole, developmental times were barely longer on *S. richteri* (0.2, 6.2, and 2.9% for larval, pupal, and total times, respectively) compared with *S. invicta*. Male and female developmental times were not significantly different by host ( $F_{1,547} = 1.27$ ,  $P = 0.26$ ). The relationship between larval and pupal times were significantly correlated in all host by sex combinations, although variation explained by this association was low ( $F_{1,157} = 5.84$ ,  $r^2 = 0.03$ ,  $P = 0.017$ ;  $F_{1,295} = 22.75$ ,  $r^2 = 0.07$ ,  $P < 0.0001$  for females and males reared on *S. invicta*, and  $F_{1,29} = 5.05$ ,  $r^2 = 0.15$ ,  $P = 0.03$ ;  $F_{1,68} = 12.23$ ,  $r^2 = 0.17$ ,  $P = 0.0009$  for females and males, respectively, reared on *S. richteri*).

**Temperature Effects.** Temperature, as expected, had a substantial effect on developmental times for all stages. Considering phorids from both hosts together, we found that the lengths of the larval and pupal periods, as well as the total developmental time, were 23.1, 30.7, and 27.4% longer, respectively, at 22°C compared with 28°C ( $F_{1,546} = 36.4$ ,  $P < 0.0001$ ;  $F_{1,546} = 308.7$ ,  $P < 0.0001$ ;  $F_{1,546} = 249.1$ ,  $P < 0.0001$  for larval, pupal, and total development times, respectively). Analyzing data from each host species separately, we found the same pattern for both *S. richteri* and *S. invicta* (Table 1); significantly longer times at 22 than at 28°C for larval, pupal, and total developmental times ( $F_{1,453} = 45.7$ ,  $P < 0.0001$ ;  $F_{1,453} = 230.41$ ,  $P < 0.0001$ ;  $F_{1,453} = 182.5$ ,  $P < 0.0001$  for larval, pupal, and total development times on *S. invicta*, respectively, and  $F_{1,100} = 20.5$ ,  $P < 0.0001$ ;  $F_{1,100} = 91.6$ ;  $F_{1,100} = 74.2$ ,  $P < 0.0001$  for larval, pupal, and total development times on *S. richteri*, respectively). Developmental times, according to temperature, did not differ by sex ( $F_{1,545} = 0.2$ ,  $P = 0.65$ ;  $F_{1,545} = 1.18$ ,  $P = 0.26$ ;  $F_{1,545} = 0.08$ ,  $P = 0.78$  for larval, pupal, and total times, respectively). The one significant host-temperature contrast we found was that pupae in *S. invicta* developed faster than those in *S. richteri* at 22°C ( $t = 2.77$ ,  $N = 416$ ,  $P = 0.0003$ ).

**Host Size Preference.** *Pseudacteon obtusus* elected the same worker head size class regardless of host species or worker size class offered ( $Z = 0.262$ ,  $N_{ig} = 428$ ,  $N_{ig} = 94$ ,  $P = 0.79$ ; and  $Z = 1.381$ ,  $N_{im} = 181$ ,  $N_{rm} = 59$ ,  $P = 0.17$ , for big and mixed *S. invicta* and *S. richteri*, respectively, elected median head sizes 0.81–0.84; Figs. 1 and 2). The phorid sex ratio (females to males) was significantly higher in the mixed class than in the big class ( $Z = 2.7$ ,  $N_{ig} = 35$ ,  $N_{rm} = 17$ ,  $P = 0.0063$ , host data pooled). When big sized ants were

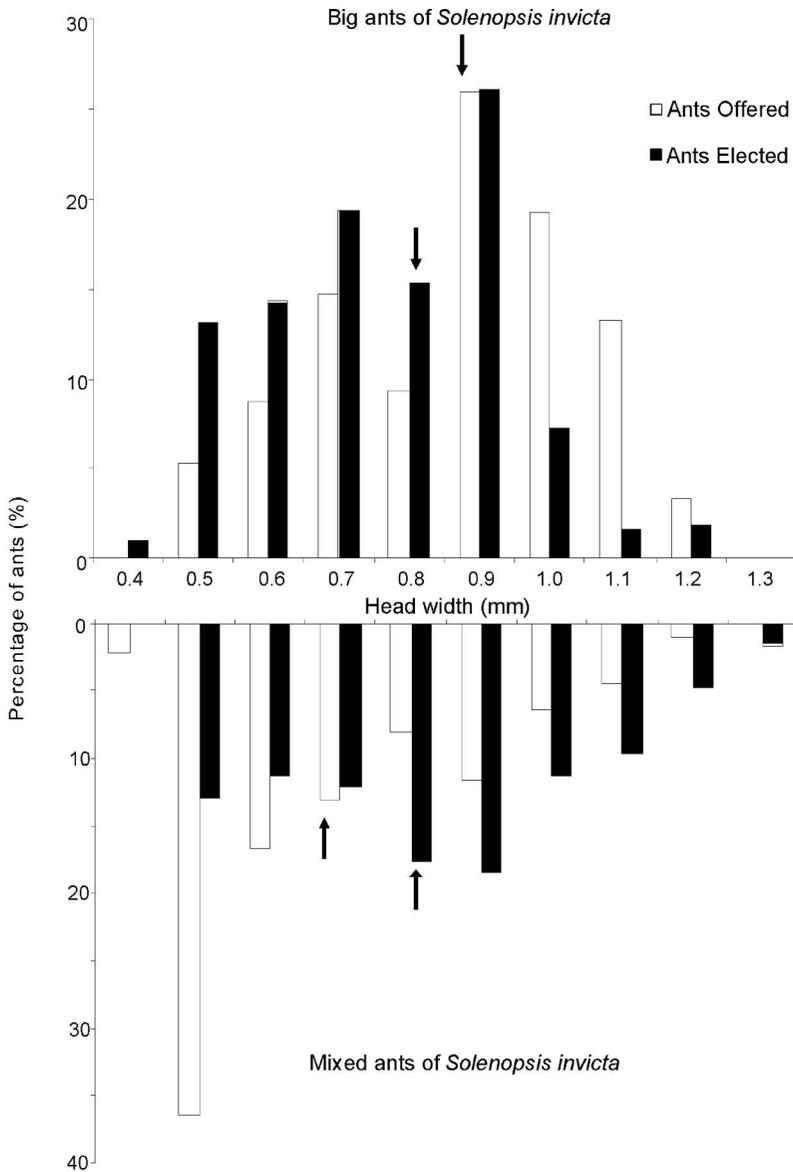
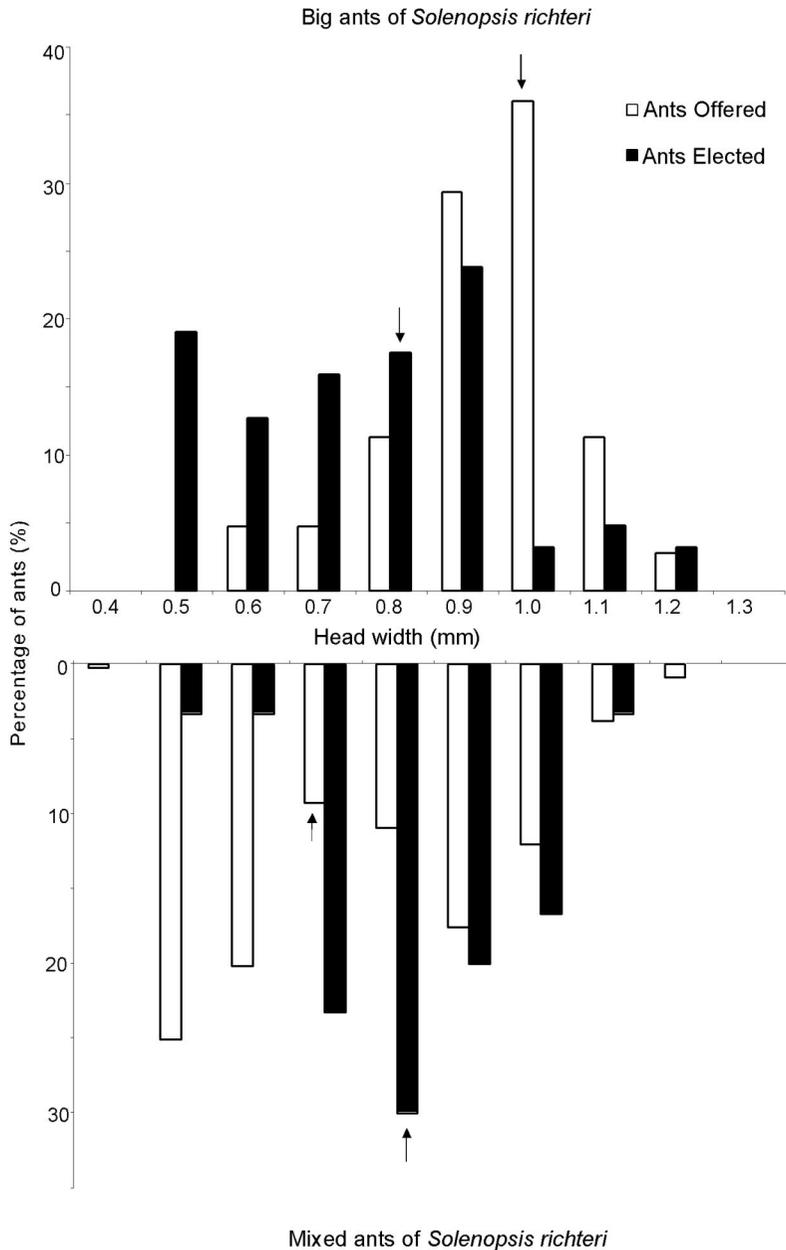


Fig. 1. Percentages of sieved big (top) and mixed-size (bottom) *S. invicta* ants discriminated by size classes offered to and elected by *P. obtusus*. Arrows point to the median size classes. There were significant differences between the median size of ants offered and elected in both treatments.

offered, the sex ratios were 102:212 ( $N = 30$ ) and 16:46 ( $N = 9$ ), whereas they were 49:80 ( $N = 5$ ) and 14:17 ( $N = 8$ ) when mixes were available for *S. invicta* and *S. richteri*, respectively.

The size distribution of heads of *S. invicta* workers parasitized by *P. obtusus* did not differ from that offered ( $\chi^2 = 0.03$ ,  $N = 464$ ,  $P = 0.97$ ;  $\chi^2 = 0.0$ ,  $N = 575$ ,  $P > 0.99$ ; Kolmogorov-Smirnov tests; Fig. 1). Our data for size classes offered to *S. richteri* did not permit a comparison with this test (Fig. 2). Median head widths of ants offered differed significantly from those parasitized: *P. obtusus* elected to attack smaller ants when

big ants were offered ( $Z = 6.21$ ,  $N_{of} = 150$ ,  $N_{el} = 314$ ,  $P = 0.00$ ;  $Z = 6.77$ ,  $N_{of} = 150$ ,  $N_{el} = 63$ ,  $P = 0.00$ ; elected 0.81 mm for both species; offered 0.93 and 1.01 mm for *S. invicta* and *S. richteri*, respectively) but elected larger ants when mixed size ants were offered ( $Z = 7.06$ ,  $N_{of} = 450$ ,  $N_{el} = 125$ ,  $P = 0.01$ ;  $Z = 2.45$ ,  $N_{of} = 450$ ,  $N_{el} = 30$ ,  $P = 0.01$ ; elected 0.84 mm for both species; offered 0.74 and 0.75 mm for *S. invicta* and *S. richteri*, respectively). Elected sizes did not differ between hosts ( $Z = 0.25$ ,  $N_i = 314$ ,  $N_r = 63$ ,  $P = 0.80$ ; and  $Z = 0.21$ ,  $N_i = 125$ ,  $N_r = 30$ ,  $P = 0.84$  for big and mixed classes, respectively).



**Fig. 2.** Percentages of sieved big (top) and mixed-size (bottom) *S. richteri* ants discriminated by size classes offered to and elected by *P. obtusus*. Arrows point to the median size classes. There were significant differences between the median size of ants offered and elected in both treatments.

#### Size Distribution According to Sex of Parasitoids.

For both host species, the size of ant heads for those that gave rise to females was significantly larger than those yielding males or nonviable pupae ( $F_{1,509} = 81.1$ ,  $P < 0.0001$ ;  $F_{1,150} = 20.2$ ,  $P < 0.0001$  for *S. invicta* and *S. richteri*, respectively). This result was obtained with both big and mixed ant size class treatments ( $F_{1,352} = 55.67$ ,  $P < 0.0001$ ;  $F_{1,58} = 9.8$ ,  $P < 0.0001$  for *S. invicta* and *S. richteri* big, respectively;  $F_{1,352} = 55.67$ ,  $P < 0.0001$ ;  $F_{1,58} = 10.9$ ,  $P < 0.0001$  for *S. invicta* and

*S. richteri* mixed, respectively). The head sizes that gave rise to males were significantly smaller than those that contained nonviable immatures in *S. invicta* ( $F_{1,391} = 23.61$ ,  $P < 0.0001$ ) but not for *S. richteri* ( $F_{1,120} = 1.64$ ,  $P = 0.18$ ). Males came from all size classes of ants offered, whereas females only came from larger ants (Fig. 3). The mean size of males ( $0.38 \pm 0.06$  mm), as measured by thoracic width, was significantly smaller ( $F_{1,229} = 124.1$ ,  $P < 0.0001$ ) than that of females ( $0.45 \pm 0.04$  mm).

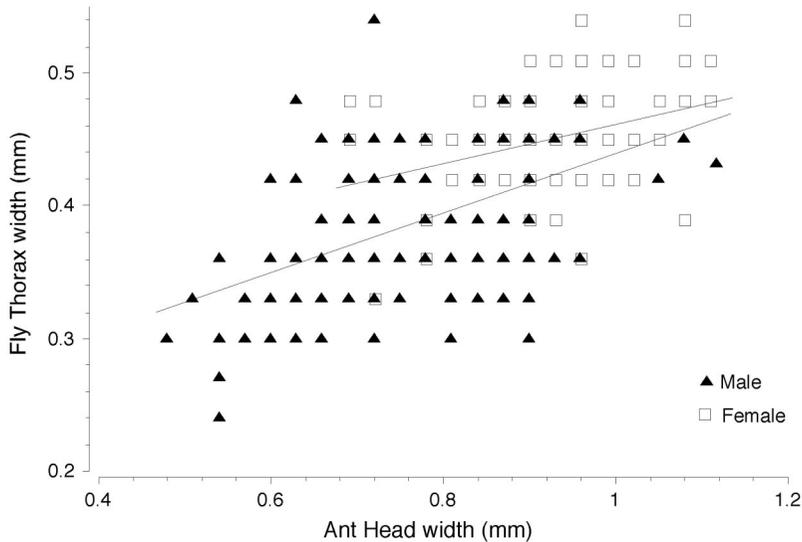


Fig. 3. Regressions of host ant head size (mm) against the resulting fly thoracic width (mm). Fly thoracic widths and the head sizes of host ants were both significantly larger for females than males. Male thoracic widths and host ant head sizes overlap the size distribution of the females. The equations are for males, thoracic width =  $0.28 \times$  head width +  $0.17$ ,  $r^2 = 0.44$ ,  $P = 0.0001$ , and for females, thoracic width =  $0.15 \times$  head width +  $0.31$ ,  $r^2 = 0.16$ ,  $P = 0.0002$ . Ants used in this analysis were from the *S. invicta*,  $22^\circ\text{C}$  treatment.

**Size of Host Ant, Flies, and Developmental Times.** Larval, pupal, and total development times did not differ significantly when adult parasitoids were offered big ants or a mixture of ant sizes ( $F_{1,530} = 1.27$ ,  $P = 0.54$ ;  $F_{1,530} = 0.17$ ,  $P = 0.68$ ;  $F_{1,530} = 0.04$ ,  $P = 0.84$  for larval, pupal, and total development, respectively). Given that developmental time was strongly affected by temperature, we ran a step-wise regression of the relation between developmental times and head sizes within the group of adults that were reared on *S. invicta* at  $22^\circ\text{C}$ , our largest class sample (Table 1). This exploratory analysis indicated that, in addition to ant head size ( $F_{1,229} = 5.17$ ,  $P = 0.0001$ , partial  $r^2 = 0.44$ ) and fly sex ( $F_{1,229} = 6.74$ ,  $P = 0.0001$ , partial  $r^2 = 0.05$ ), total development time was significantly related to fly size ( $F_{1,229} = 2.7$ ,  $P = 0.0075$ , partial  $r^2 = 0.016$ ). Within each sex, fly size as measured by thoracic width was significantly related to host ant head size ( $t = 13.44$ ,  $N = 228$ ,  $P < 0.0001$ ;  $t = 6.93$ ,  $N = 148$ ,  $P < 0.0001$  for all males and females, respectively; Fig. 3).

## Discussion

This is the fifth in a series of studies that provides a comparative analysis of development for a *Pseudacteon* species on both fire ant species (*S. invicta* and *S. richteri*) that are exotic pests in North America (see Porter et al. 1997, Porter and Briano 2000, Folgarait et al. 2002a, b). *P. obtusus* was able to successfully develop on both species of imported fire ants. Production and survivorship of this population of *P. obtusus* was higher on *S. invicta* than on *S. richteri*, which is consistent with data reported by Folgarait et al. (2002a) for four species of *Pseudacteon*, including *P. obtusus* from Buenos Aires and Corrientes. Populations of

*P. obtusus* have greater abundances when associated with *S. invicta* than *S. richteri*, and the difference in host performance may help explain these population patterns. While *P. obtusus* developmental times were similar between hosts, the host dependent difference in production indicates that this population may not do as well if introduced into the United States for management of the black imported fire ant.

The performance of *P. obtusus* differed little with respect to developmental times and resulting adult sizes of both male and females when reared on *S. invicta* or *S. richteri*. Folgarait et al. (2002a) found, however, that total development of *P. obtusus* was significantly longer on *S. invicta* than on *S. richteri* at  $22^\circ\text{C}$ . We also found a significant difference between hosts with our much larger data set, although only in pupal developmental time and only at the  $22^\circ\text{C}$  temperature regimen used in Folgarait et al. (2002a). Those development time values are similar to those reported here.

Few studies contrast developmental times of *Pseudacteon* across temperatures (Morrison et al. 1997, Folgarait et al. 2002a, b, this study). Porter et al. (1995b) hazarded a prediction for *P. litoralis*, based on host brood developmental patterns, that a six degree increase in temperature might be represented by "perhaps more than twice as fast" an increase in fly development. These studies suggest only a slightly more conservative estimate of the effect of temperature on *Pseudacteon* development. Developmental rate for *P. obtusus* in this study, for instance, was increased by an average of 27% over the  $6^\circ$  increase in temperature, depending on stadia, host, and sex combination). *P. browni* and *P. tricuspis*, likewise, had increases in egg-larval developmental rates of 25 and 37% when

reared at 30 versus 25°C (Morrison et al. 1997). Developmental rates for *P. cultellatus* (Folgarait et al. 2002b) reared at temperatures that differed by only 3–4° (25 versus 22°C and 29 versus 25°C) varied by ≈11–14%, whereas Folgarait et al. (2002a) found differences in developmental rates of ≈15% for several species of *Pseudacteon* reared at 3–5°C (22, 25, and 27°C) contrasting temperatures. Taken together, these studies suggest that, for *Pseudacteon*, there is an increase in developmental rate of around 2–9% (compounding) for each degree Celsius increase in temperature, within the 20–30°C range. Our study had values that ranged from 2.2 to 7.5% per 1°C (mean, 5.2%), whereas the maximum given by Porter et al. (1995b) was for at least a 10.9% per 1°C increase. It is interesting that in the available studies, pupal developmental times increased at a faster rate than did larval developmental times.

The laboratory performance of *P. obtusus*, as measured by survivorship, was better at the lower temperature treatment of 22 than 28°C. This temperature corresponds well with the average temperature found in Buenos Aires (Schwerdtfeger 1976) around the time of peak incidence of this species in this area (Folgarait et al. 2003). While we do not understand the phenological details of this fly in Corrientes, the average annual temperature is ≈21.3°C (worldclimate.com), suggesting that the 22°C may parallel normal conditions for this fly in that area as well.

Other studies indicate other *Pseudacteon* species may also have better survivorship at lower temperatures. Porter et al. (1997), for instance, reported higher but not significantly higher survivorship for tropical Brazilian *P. tricuspis* at 24 than at 30°C. *P. cultellatus* reared in Folgarait et al. (2002b) also had increased pupal survivorship of *P. cultellatus* when kept at 22 versus 25°C (Folgarait et al. 2005). Because we found that developmental time of *P. obtusus* increased marginally with increasing temperature but that this came at the considerable expense of increased mortality, we have taken to rearing our *P. obtusus* at 22°C. Folgarait et al. (2002b) were unable to determine an interaction between developmental time and survivorship in *P. cultellatus* and suggested that a faster developmental time was desirable for mass-rearing. We amend their suggestion to incorporate finding the temperatures under which mass rearing of *Pseudacteon* can be adjusted to obtain the optimal balance between increased production by developmental rate and survivorship.

*Pseudacteon* species show considerable variation in sexual dimorphism and environmental sex determination (Morrison et al. 1999). As with many insects, females are, on average, larger than males. This pattern is intensified in *P. tricuspis* and *P. litoralis*, where there is little overlap in size of males and females and size of their host head sizes (Morrison and Gilbert 1998). In contrast, Folgarait et al. (2002b) found no difference between mean male and female size in *P. cultellatus* or a concordant difference between the respective host head widths of the sexes. Sex in *P. cultellatus* was not determined by host size. Our study of *P. obtusus* shows an intermediate situation between these taxa. Male

*P. obtusus* were significantly smaller than females but their observed size range overlapped the entire distribution seen for females (Fig. 2). In other words, the size ranges of female *P. obtusus* and their host heads were subsets of those of the males. Host size only restricts female production in *P. obtusus*.

We found that a mixture of host sizes produced a greater proportion of females than did the big host size class offered. This result is contrary to our expectations based on other larger *Pseudacteon*, such as *P. tricuspis* and *P. litoralis* (Morrison and Gilbert 1998). Our laboratory mass-rearing of *P. tricuspis*, for example, involves sieving ants to obtain a suitable fraction of large ants to increase female bias in the sex ratio. With respect to mass-rearing of *P. obtusus*, these observations suggested that sieving for larger workers would be contraindicated. The first author has subsequently found, however, that mixtures of “big” and “small” ants (not including the medium class) produce more female-biased sex ratios than other combinations of ant size classes. Our interpretation is that the strong contrast in size among the ants in this sieved mix may facilitate host choice in *P. obtusus*. Field observations of *P. obtusus* (P.J.F. and J.W.P., unpublished observations) indicate that females spend more time tracking their progeny’s host ants before ovipositing than do other species, such as *P. nocens* or *P. cultellatus*. This attention to the host may allow the fly to make a finely tuned approximation of host size or quality that would increase the likelihood of progeny becoming female.

The implications of these host size-dependent sex ratios on biological control introductions are not obvious. Gilbert and Patrock (2002) discussed the challenges of introducing *P. tricuspis* in Texas where polygyne *S. invicta* predominates. The relatively small ants of this social form produce highly male-biased sex ratios, and as a result, relatively low effective population sizes, in this phorid species. Female *P. obtusus* were produced from ants that are smaller (this study) than that documented for *P. tricuspis* (Morrison and Gilbert 1998) and within a size class of polygyne fire ants that is much larger (Greenberg et al. 1985) than that expected for *P. tricuspis*. These observations suggest that production of females in *P. obtusus* may not be as constrained by the small ants typically found in *S. invicta* polygyne populations.

Variation in adult size among populations of *P. obtusus* is also very striking. Our study was based on flies from Corrientes Province in Northern Argentina. Here, females are significantly larger (25% larger on average) than that of Brazilian *P. obtusus* discussed in Orr et al. (1997) and up to 40% larger than a population of small *P. obtusus* found in Santiago del Estero Province in Western Argentina (P.J.F., personal observation). These size differences are the largest observed for different populations of a single species of *Pseudacteon* and probably for most Phoridae (P.J.F., unpublished data). The differences in body sizes across populations suggested that these populations might be represented by cryptic species. Amplified fragment length polymorphism (AFLP) molecular analyses of

larger and smaller flies within one population in Santiago del Estero show that these size morphs are genetically distinguishable (M. Cronfurst and L.E.G., unpublished data). This study includes data only for the larger *P. obtusus* biotype.

*Pseudacteon obtusus* has a broad geographical distribution that stretches across a very large set of temperature and humidity gradients in Brazil to Argentina (16–34° S and 49–57° W, Folgarait et al. 2005). In addition to its climatic range, *P. obtusus* is hosted by a number of different *Solenopsis* fire ants (Folgarait et al. 2002a). *S. invicta* is a common host in Corrientes, the source of our flies, as well as in Western Argentina (P.J.F., unpublished data). *S. richteri* is a host in Buenos Aires at the RECS (Folgarait et al. 2003) and other locations but is not common at any known sites with this host (P.J.F., unpublished data). In fact, Folgarait et al. (2003) found *P. obtusus* to be the least abundant of all six *Pseudacteon* at the RECS. However, in Corrientes, on *S. invicta*, *P. obtusus* is the most commonly found and abundant *Pseudacteon* (P.J.F., unpublished data). Its temporal activity pattern is very wide; it can be found ovipositing on ants from morning until twilight, and depending on the locality, can be found across most (Folgarait et al. 2003) or all of the months of the year (P.J.F., unpublished data). *P. obtusus* is found attracted to ant trails of *S. richteri* around Buenos Aires (Bruzzone 2004) and *S. invicta* in Mato Grosso Brazil (Williams and Banks 1987), as well as to disturbed mounds (Folgarait et al. 2003). In Western Argentina, as well, *P. obtusus* mixes its foraging strategy (P.J.F. and G. Azzimonti, unpublished data), such that it is not easily placed in the categories of Orr et al. (1997) of mound versus trail phorids. While individual flies do not seem to mediate fire ant activity as strongly as some other *Pseudacteon* species (Folgarait et al. 2002, Wuellner et al. 2002, P.J.F., unpublished data), more than one fly at a time may be found attacking ants at a food resource (Williams and Banks 1987, Patrock, Unpublished).

*Pseudacteon obtusus* has a number of characteristics that advocate for its use as a biological control agent throughout the exotic ranges of the imported fire ants. From a safety perspective, *P. obtusus* is considered to be highly host specific (Morrison and Gilbert 1999) to the *saevissima* group of *Solenopsis* (Trager 1991). From an effectiveness perspective, *Pseudacteon* are considered to affect fire ants primarily by disrupting fire ant foraging. *Pseudacteon obtusus* is reliable in this respect, in that it has a mixed host-finding strategy, being commonly found on ant trails, as well as disturbed mounds. In Santiago del Estero, Argentina for instance, it can be one of the most common and influential phorids attacking *S. invicta* on trails (R.W.P., unpublished data). Additionally, populations of this fly are found in areas with climates that resemble many in the southern United States. Mortality results in our study indicated that the Corrientes population may not perform well in the extremes of south Texas, although in other regions of Texas, this population may do better. From our undergoing field observations, *P. obtusus* from the arid Chaco of Santiago del Estero

are likely to be a better choice for applied use in hotter and drier areas of Texas. In areas where cold temperatures may be limiting, *P. obtusus* from the Buenos Aires area may be given priority as they undergo an overwintering diapause to weather cold temperatures (Folgarait et al. 2003). We would consider the introduction of *P. obtusus* to be complementary to other *Pseudacteon* currently released, such as *P. tricuspis* (Gilbert and Patrock 2002, Porter et al. 2003) and *P. curvatus* (Graham et al. 2003), or those planned to be released (*P. cultellatus*), because it develops in both imported fire ant species, as well as in a wide range of host sizes, including size classes that are intermediate to those attacked by the mentioned taxa. Finally, we have been able to rear this species for a number of continuous generations in the laboratory, suggesting that we will soon be able to mass rear this species for further studies and field releases.

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