

# Influence of Adult Size on Mate Choice in the Solitary and Gregarious Parasitoids, *Cotesia marginiventris* and *Cotesia flavipes*

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**Abstract** Female and male mate choice in relation to adult size were examined for the solitary and gregarious parasitoids, *Cotesia marginiventris* (Cresson) and *Cotesia flavipes* Cameron, respectively. In addition, male precopulatory behaviors were observed for evidence of male competition or a large-male advantage in mate acquisition. Male parasitoids are not known to offer female mates direct benefits, but females that mate high quality males may obtain indirect benefits, such as offspring that are more successful in obtaining mates. Female choice experiments for *C. marginiventris* found that large males approached females first more frequently than small males, and that females mated large males significantly more often than small males. Male choice experiments for *C. marginiventris* did not demonstrate a male preference for female size. In contrast, female choice experiments with *C. flavipes* found that females mated equally with large or small males, while male choice experiments showed that males attempted copulation and mated more frequently with smaller females. Male competition was not observed in the gregarious species *C. flavipes*, but competition in this gregarious parasitoid could be moderated by dispersal.

**Keywords** Courtship behavior · female choice · indirect benefit · male competition · Braconidae · male choice

## Introduction

Mate choice in insects can occur from female or male choice of mating partners, or as an outcome of male competition. Females are predicted to be the selective sex

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when choosing mates because they typically need to mate only once to fertilize their eggs (Thornhill and Alcock 1983; Davies 1991; O’Neil 2001). Thus, males may have evolved to increase their attractiveness. For example, males may provide direct benefits to mates, such as territory or nutrients that increase female fertility or longevity, or indirect benefits, such as superior genetic quality of offspring or more attractive sons (Jones et al. 1998). Male competition may also occur prior to mating, and is predicted to occur where resources such as females are concentrated (Thornhill and Alcock 1983; Davies 1991).

In the case of parasitoids, mate choice can also be influenced by the spatial location of hosts in the field (Thornhill and Alcock 1983; Davies 1991; Godfray 1994, Godfray and Cook 1997). Solitary parasitoids that attack dispersed hosts have males which must search for mates, and these males may compete to acquire females (Godfray 1994). Parasitoid males are not known to offer females direct benefits or resources, such as spermatophores or nuptial gifts, to entice females to mate (Godfray 1994). However, solitary female parasitoids could choose mates to receive indirect benefits, such as larger offspring or more attractive sons, as in other insects (Capone 1995; Greenfield 2002). In gregarious and quasigregarious parasitoids, emerging adults may mate locally with siblings; female choice may be unlikely as there is little to gain genetically. In contrast, males from gregarious clutches may compete directly for female mates, which are concentrated at the emergence site, to increase their mating success (Hardy 1994; Godfray and Cook 1997). Choosiness for mates may be greater for parasitoids, either solitary or gregarious, that exhibit complementary sex determination in order to prevent inbreeding and production of diploid males (Ode et al. 1995; de Boer et al. 2007).

Few studies have examined female or male mate choice in parasitoids (Godfray and Cook 1997; Quicke 1997; Gu and Dorn 2003; Martel et al. 2008). Females of the gregarious species *Bracon hebetor* Say (Braconidae) mated more frequently with non-siblings than with siblings (Ode et al. 1995). In contrast, females of the gregarious parasitoid *Cotesia glomerata* (L.) showed no mating preference for sibs or non-sibs (Gu and Dorn 2003). In both solitary and gregarious parasitoids, a large-male advantage in mate acquisition has been documented (Eggleton 1990; Lampson et al. 1996; Abe et al. 2005), as have competitive mating tactics such as mate stealing (Field and Keller 1993b). Other studies found no large-male precedence or advantage in mating (Crankshaw and Mathews 1981; Suzuki and Hiehata 1985; Antolin and Strand 1992; Cheng et al. 2003). Additional laboratory and field investigations may provide insight to mate choice processes (Godfray and Cook 1997).

This study examined mate choice in two parasitoids with contrasting mating systems, the solitary parasitoid, *Cotesia marginiventris* (Cresson), and the gregarious parasitoid, *Cotesia flavipes* Cameron. The first parasitoid, *C. marginiventris*, is an endoparasitoid of first and second instar larvae of Noctuidae (Lepidoptera) (Boling and Pitre 1970; Tillman 2001). Its hosts, such as *Spodoptera frugiperda* (J. E. Smith), do not occur in aggregations, so adult parasitoids must disperse upon emergence to locate mates. Male development time is 1 day shorter than that of females, and males are attracted to a female pheromone (Joyce, unpublished data). Offspring sex ratios vary from 60–70% males (Kunnalaca and Mueller 1979; Ramírez-Romero et al. 2007) to 40% males (Novoa and Luna 1996). The second species, *C. flavipes*, is a gregarious parasitoid that attacks stem boring larvae of

Pyralidae (Lepidoptera), and exhibits considerable local mating within a brood (Bernal, unpublished data). Broods of ~40 individuals emerge from a single larval host and have a female-biased sex ratio, with typically four or five females per male (Wiedenmann et al. 1992; Kimani and Overholt 1995; Potting et al. 1997). Males mate with multiple females, but females typically mate only once, and reject males after mating (Arakaki and Ganaha 1986). Courting males of both *C. flavipes* and *C. marginiventris* fan their wings when in close range of females (Sivinski and Webb 1989; Kimani and Overholt 1995), producing a low amplitude sound and substrate vibrations (Joyce et al. 2008; Joyce, personal observation). Females of both species signal acceptance of male copulation attempts by remaining stationary and allowing the male to mount and copulate. In contrast, females reject males by jumping away and producing a buzzing sound with their wings. Individuals of both species are small ( $\leq 3$  mm body length), and have no notable color patterns or markings that might provide visual cues to potential mates.

Specifically, the goal of this study was to test whether mate choice for large or small mates occurred in *C. marginiventris* and *C. flavipes*. Mate choice was examined at the courtship level to determine if mate size influenced mating success. Male precopulatory behaviors were also examined for evidence of direct male competition or a large-male advantage in mate acquisition. Female choice could occur in either the solitary or the gregarious parasitoid species, but was expected to be less likely in the gregarious parasitoid due to genetic relatedness of siblings. Males of both species were not expected to be selective with respect to female mate size. Male competition was expected to be more likely in the gregarious parasitoid, *C. flavipes*, than in the solitary parasitoid, *C. marginiventris*, while a large-male advantage was expected in both species.

## Materials and Methods

### Insects

*C. marginiventris* were reared on *S. frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). Adult *S. frugiperda* were maintained in 2.4 L plastic containers with a 20% sucrose solution, lined with paper towels which served as an oviposition substrate, at  $27 \pm 2^\circ\text{C}$ ,  $60 \pm 5\%$  relative humidity (RH), and a 15:10 (L:D) photoperiod. Paper towel strips (4×6 cm) with eggs were placed inside 1 L glass jars, and emerging larvae fed on a corn earworm diet (Bio-Serv, Inc, Frenchtown, NJ, USA). To parasitize larvae, one adult male and female *C. marginiventris* (0–48 h old) were placed in a 24 mL glass vial with 20 *S. frugiperda* second instar larvae and larval diet for 48 h. Parasitized larvae were then transferred to 10 mL plastic cups with artificial diet, and incubated for approximately 7 days until parasitoid cocoons appeared. Individual cocoons were then placed singly in 1 mL (1/4 dram) glass vials and closed with cotton, so that emerging adults were virgin for experiments. All *C. marginiventris* used in trials were stored overnight at  $15^\circ\text{C}$  before mating trials were conducted.

*C. flavipes* was reared on *Diatraea saccharalis* (F.) (Lepidoptera: Pyralidae) in a manner similar to *S. frugiperda*. Adult moths were held in 2.4 L plastic containers

with wax paper as an oviposition substrate. Wax paper strips (4×6 cm) with eggs were placed in 1 L glass mason jars with diet for emerging larvae (Martinez et al. 1988). To parasitize larvae, single mated female *C. flavipes* (0–48 h old) were placed in 10 mL plastic cups with two larvae (third–sixth instar) and artificial diet. Approximately 1 week later, parasitoid cocoons were collected and isolated individually in glass vials as described above.

## Plants

Trials involving *C. marginiventris* were conducted on a leaf of a young maize plant (*Zea mays* L.) in an arena. Previous work showed that a low proportion (<30%) of this species mated in glass vials or plastic Petri dishes, and that using a plant as a mating substrate substantially increased the proportion mated (Joyce et al. 2008). Maize plants were grown from Pioneer<sup>®</sup> seed 34A55 (Johnston, IA, USA), planted in Miracle Grow<sup>®</sup> (Marysville, OH, USA) potting soil in plastic pots (13 cm diameter×12 cm height) in a greenhouse with natural light ~15:10 (L:D), 30±5°C, and 50–90% RH. Plants used for experiments were 30–40 cm tall and had five leaves.

## Selecting Large and Small Parasitoids

Each trial used newly emerged virgin adult male and female parasitoids. A binocular microscope fitted with a lens micrometer was used to grossly estimate parasitoid body length, and wasps were grouped into small or large size classes. The difference between large or small parasitoids was visually apparent to the unaided eye, and was later found to represent a ~10% size difference. The right hind tibia length (RHTL) was measured in mm for each parasitoid after each experiment, and was used as a proxy for adult size in all experiments. Parasitoid individuals from these large and small size classes were used in the mate choice trials described below.

### *Experiment 1: Mate Choice by C. marginiventris Females*

Each trial was conducted in the laboratory between 08:00 and 10:00 h, at 25±2°C, 45–60% RH. The arena for each trial consisted of a 160 mL plastic vial (4.8 cm diameter×8.4 cm length) covering one leaf of a maize plant, which remained attached to the plant. The vial arena was placed around the leaf, but not touching it, and was supported by attaching the vial to the stem of the plant. All *C. marginiventris* used in these studies were younger than 48 h old. Each parasitoid, arena, leaf, and plant were used only once. Two males, one large and one small, were released in the arena, followed immediately by a female.

Male precopulatory behaviors were recorded to determine if there was direct male–male competition or a large-male advantage. The frequency and the latency (time elapsed from the start of the experiment to the first display of a behavior) in seconds of the following male precopulatory behaviors were recorded for the large and small male in each trial: (1) first wing fanning, (2) first approach to a female, and (3) first attempted copulation. These were the only male precopulatory behaviors observed. The copulation latency (time elapsed from the start of the experiment until

copulation began), copulation duration, and whether the large or small male mated first with the female were recorded. After mating was complete (or 15 min if no mating occurred), each insect was collected into separate vials. A total of 43 trials were conducted, with females mating in 29 trials. Following each trial, adults were killed by freezing, and the RHTL was measured for both males and the females.

#### *Experiment 2: Mate Choice by C. marginiventris Males*

Vial arenas and maize plants, as described above, were used in these trials. For each trial, a large and a small female were placed in the arena enclosing a maize leaf, followed immediately by a male. The following precopulatory behaviors were recorded for males as they first displayed each behavior to a large or small female: (1) first wing fanning, (2) first approach to a female, and (3) first attempted copulation. In male choice experiments, display of these male precopulatory behaviors toward females was used as a measure of male preference for large or small females. Copulation latency, copulation duration, and whether the male mated the large or small female were recorded. After mating, or at the end of the trial (15 min), each insect was collected into a separate vial for later measurement of its RHTL. A total of 26 trials were conducted, and males mated in 19 trials. The RHTL was measured for all parasitoids.

#### *Experiments 3 and 4: Mate Choice by C. flavipes Females and Males*

The trials with *C. flavipes* were conducted as described for *C. marginiventris*, with the exception that trials were conducted in 24 mL glass vials (2.3 cm diameter×9 cm length). Each parasitoid and arena were used only once. All *C. flavipes* used in these trials were younger than 24 h old. In the female choice experiment, consisting of a large and small male and one female, the first display of each of the male precopulatory behaviors (described above for *C. marginiventris*) by large and small males toward the female was recorded. In the male choice experiment, male precopulatory behaviors were displayed to a large or small female, but data were not collected for wing fanning. In both experiments, the copulation latency and duration were recorded, as well as the mating frequency with large or small mating partners. In the female choice experiment (Experiment 3), the female mated in 27 of the 36 trials. In the male choice experiment (Experiment 4), the male mated in 20 of the 25 trials. The RHTL was measured in all parasitoids.

#### Statistical Analyses

##### *Experiment 1: Mate Choice by C. marginiventris Females*

Data from all trials in which mating did not occur were excluded from analyses. The frequency of each male precopulatory behavior for large and small males was compared using Chi-square tests, while the latency to each large or small male behavior was compared using Mann–Whitney *U* tests. Chi-square tests compared the success of the first attempted copulations by large or small males, as well as the mating frequencies of large or small males. Fisher's exact tests were applied for

frequencies smaller than 5. The RHTL was compared between large and small males using *t*-tests. In addition, paired *t*-tests compared the mean RHTL difference of the copulating male and female pair (=mated male RHTL–female RHTL) to the difference in RHTL of the noncopulating male and female (=unmated male RHTL–female RHTL). All *t*-tests were two tailed. Mean copulation durations, as well as mean copulation latencies, were compared between large and small males using Mann–Whitney *U* tests. Large-sample Mann–Whitney *U* tests were used for categories with more than ten observations, with corrections for tied data, and results were reported as *Z*-scores (Siegel and Castellan 1988).

### *Experiment 2: Mate Choice by C. marginiventris Males*

Statistical analyses were similar to those described (above) for mate choice by *C. marginiventris* females, but tests were applied to male behaviors toward a large or small female. The frequency of each male precopulatory behavior toward a large or small female was compared using Chi-square tests, as were frequencies of acceptance or rejection of male copulation attempts to large or small females. The RHTL was compared between the large and small females using *t*-tests, as was the RHTL difference of the male and copulating female and the RHTL difference of the male and noncopulating female. Mean copulation durations, as well as mean copulation latencies, were compared between males that mated with large or small females

### *Experiments 3 and 4: Mate Choice by C. flavipes Females and Males*

All analyses were similar to those described above for mate choice by *C. marginiventris* females and males, with one difference. In the *C. flavipes* male choice experiment, only one male mated with a large female, so copulation duration and latency could not be compared between large and small females.

Comparisons were also conducted of mean copulation durations and copulation latencies between the female choice and male choice experiments, within each species, using Mann–Whitney *U* tests. If male guarding or interference occurred in the female choice experiment, then longer copulation latencies or durations would be expected than in the male choice experiment.

All statistical analyses were conducted using Statistix (2000) or SAS (1996). All comparisons were two-tailed where applicable.

## **Results**

### Mate Choice by *C. marginiventris* Females

Large and small males were equally likely to first exhibit wing fanning towards a female (Table 1). Large males were more frequently first to approach females, but they attempted copulation as frequently as small males (Table 1). The mean latencies for these three behaviors were not significantly different between large and small males (Table 1). Female acceptance of male copulation attempts depended on male

**Table 1** The Frequency and Latency (in Seconds) to the First Display of Male Precopulatory Behaviors in the Female and Male Choice Experiments for *C. marginiventris* and *C. flavipes*

	Male precopulatory behaviors					
	First wing fan		First approach		First attempted copulation	
	First display Frequency	Latency (s) Mean±SE	First display Frequency	Latency (s) Mean±SE	First display Frequency	Latency (s) Mean±SE
<i>C. marginiventris</i>						
Female choice						
Large male	13	40.2±12.2	17	48.4±15.1	15	192.9±41.3
Small male	10	55.9±21.2	8	58.6±22.1	13	129.9±26.5
	$\chi^2=0.78,$ $P=0.38$	$Z=0.62,$ $P=0.53$	$\chi^2=6.83,$ $P=0.01$	$Z=0.38,$ $P=0.70$	$\chi^2=0.29,$ $P=0.59$	$Z=0.68,$ $P=0.50$
Male choice						
Large female	11	56.6±17.9	11	59.6±17.2	9	126.9 ±30.0
Small female	7	47.7±23.1	8	44.1±20.3	10	101.4±22.3
	$\chi^2=1.78,$ $P=0.18$	$Z=0.27,$ $P=0.79$	$\chi^2=0.95,$ $P=0.33$	$Z=0.91,$ $P=0.36$	$\chi^2=0.11,$ $P=0.75$	$Z=0.57,$ $P=0.57$
<i>C. flavipes</i>						
Female choice						
Large male	7	32.0±15.7	10	18.7 ±2.2	14	37.8±7.5
Small male	17	10.0±2.5	12	36.0±11.1	10	63.8±19.6
	$\chi^2=8.33,$ $P=0.004$	$Z=2.048,$ $P=0.04$	$\chi^2=0.36,$ $P=0.55$	$Z=0.50,$ $P=0.62$	$\chi^2=8.28,$ $P=0.25$	$Z=0.53,$ $P=0.60$
Male choice						
Large female	n/a	n/a	8	21.1±5.0	5	47.8±12.2
Small female	n/a	n/a	12	16.4±2.7	15	52.0±18.1
			$\chi^2=1.60,$ $P=0.21$	$Z=0.50,$ $P=0.62$	$\chi^2=8.10,$ $P=0.004$	$Z=0.87,$ $P=0.38$

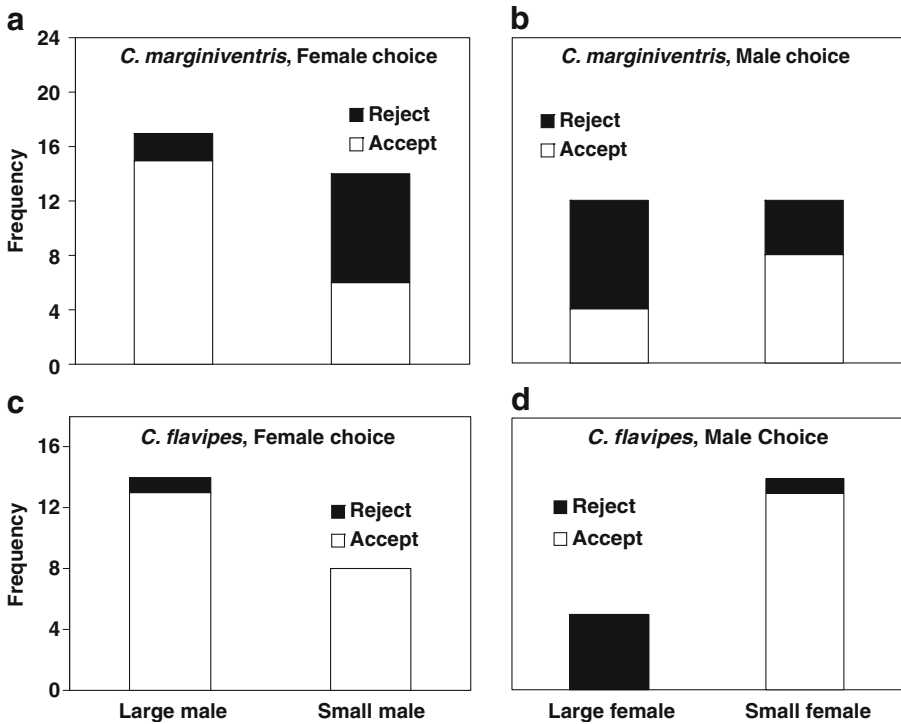
In the female choice experiments, large or small males displayed toward a female. In the male choice experiments, the single male displayed toward a large or small female. First display frequencies within each experiment were compared using a Chi-square test. The latency to the first display of each behavior was compared within an experiment using a Large Sample Mann–Whitney *U* test.

size. On the first attempted copulation, females more frequently accepted large males as mates (Fig. 1a).

Significantly more *C. marginiventris* females mated with large males than with small males (Fig. 2a). The RHTL of mated males was significantly larger than unmated males (Fig. 2b). The mean RHTL difference between the mated males and females was significantly different than for the unmated males and females (Fig. 2c), with a larger size difference between unmated males and females. Mean copulation durations were similar in large and small males (25.8±1.1 vs. 25.0±2.1 s; large sample Mann–Whitney *U*,  $Z=0.29$ ,  $N_1=19$ ,  $N_2=3$ ,  $P=0.77$ ), as were the copulation latencies (large males, 352.2±55.6; small males, 368.0±127.7 s;  $Z=0.84$ ,  $N_1=24$ ,  $N_2=5$ ,  $P=0.80$ ).

#### Mate Choice by *C. marginiventris* Males

Males fanned their wings with equal frequency towards large or small females, and approached and attempted copulation with large or small females with similar



**Fig. 1** The frequencies of female rejection or acceptance of males on the first attempted copulation in the four mate choice experiments. In *C. marginiventris*, **a** large male attempted copulations were accepted more frequently than small male attempted copulations in the female choice experiment (Fisher’s Exact Test, two-tailed,  $P=0.02$ ), while **b** large and small females accepted copulation attempts with equal frequency in the male choice experiment (Fisher’s Exact Test, two-tailed test,  $P=0.35$ ). In *C. flavipes*, **c** females accepted both large and small male copulation attempts more than expected in the female choice experiment (Fisher’s Exact Test, two-tailed,  $P<0.001$ ), and **d** small females accepted copulation attempts more frequently than large females in the male choice experiment (Fisher’s Exact Test, two-tailed,  $P=0.001$ ).

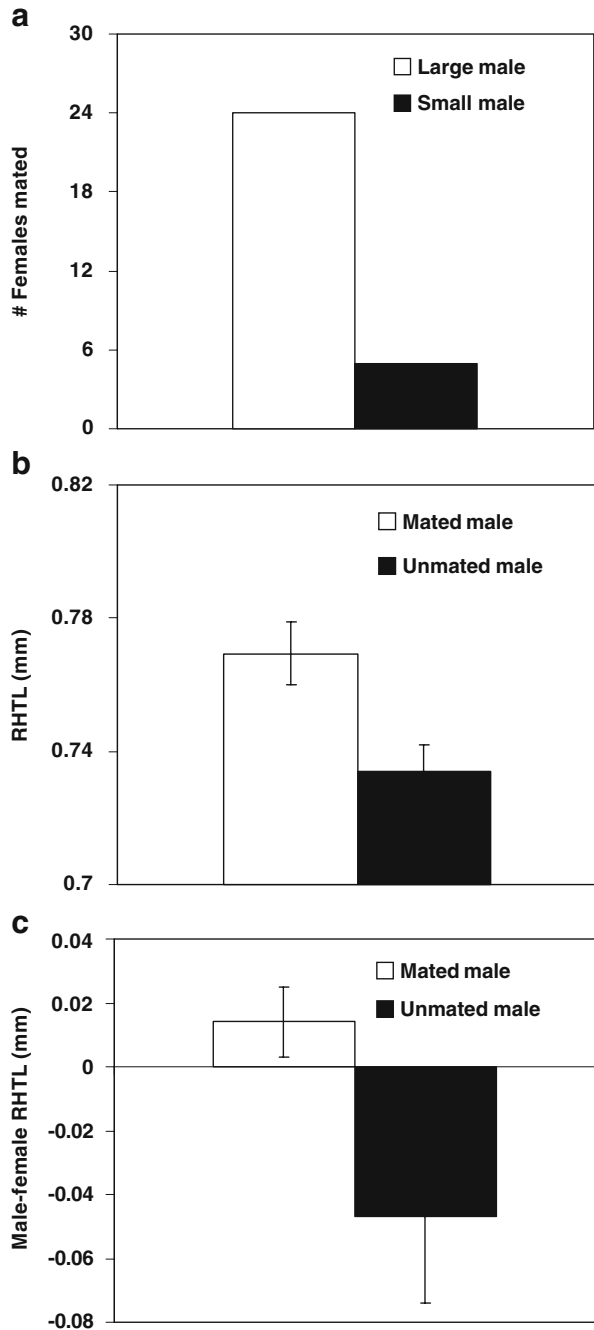
frequencies (Table 1). The latency of these three behaviors toward large and small males did not differ significantly (Table 1). Lastly, there was no significant difference in acceptance or rejection frequencies by large or small females for the first copulation attempt by males (Fig. 1b).

Male mating frequencies with large or small females were not significantly different (Fig. 3a). Mated and unmated females had RHTLs that were not significantly different (Fig. 3b). The mean RHTL difference between males and the mated female was not significantly different than the RHTL difference between males and the unmated females (Fig. 3c). Copulation duration did not differ between males mating with large ( $23.5 \pm 1.9$ ) or small females ( $21.7 \pm 1.3$  s;  $Z=1.02$ ,  $N_1=6$ ,  $N_2=11$ ,  $P=0.30$ ).

The mean copulation duration was significantly longer by several seconds in the female choice experiment, when two males were present, compared to the male choice experiment, which had only one male present (female choice,  $25.7 \pm 1.0$ ; male

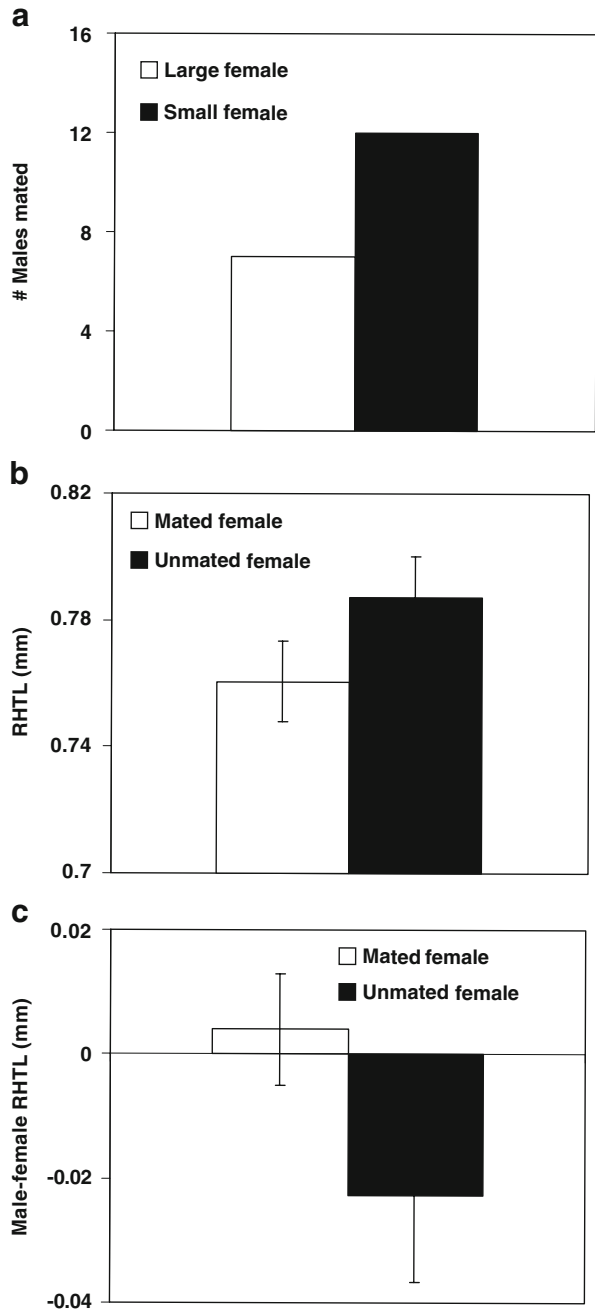


**Fig. 2** Mate choice for large or small males by *C. marginiventris* females. **a** Females mated more frequently with large than small males (Chi-square test:  $\chi^2_1 = 22.35$ ,  $P < 0.001$ ). **b** Mated males were larger than unmated males ( $t$ -test:  $t_{54} = 2.91$ ,  $P = 0.006$ ). **c** The RHTL difference between mated males and females was significantly different than the RHTL difference of unmated males and females (Paired  $t$ -test:  $t_{28} = 2.15$ ,  $P = 0.04$ ), with a larger RHTL difference between the female and unmated male. The length of the right hind tibia in millimeter (RHTL) was used as a proxy for adult size in **b** and **c**.



choice,  $22.4 \pm 1.1$  s; large sample Mann–Whitney  $U$  test,  $Z = 2.08$ ,  $N_1 = 22$ ,  $N_2 = 17$ ,  $P = 0.04$ ). In contrast, the copulation latency was similar between both experiments (female choice,  $354.9 \pm 50.1$ ; male choice,  $322.7 \pm 51.1$  s;  $Z = 0.08$ ,  $N_1 = 29$ ,  $N_2 = 19$ ,  $P = 0.93$ ).

**Fig. 3** Mate choice for large or small females by *C. marginiventris* males. **a** Males mated with large or small females with similar frequency (Chi-square test:  $\chi^2_1 = 2.65, P=0.11$ ). **b** Mated and unmated females were similar sized (*t*-test:  $t_{36}=1.49, P=0.07$ ). **c** The RHTL difference for mated males and females was not significantly different than the RHTL difference for males and unmated females (paired *t*-test:  $t_{19}=1.99, P=0.06$ ).



Mate Choice by *C. flavipes* Females

Small males were more likely than large males to first exhibit wing fanning behavior toward females, though the frequencies of first approaches and first attempted

copulations did not differ between large and small males (Table 1). Similarly, while the latency to wing fanning was significantly shorter for small males than large males, the mean latency to the first approach or attempted copulation did not differ significantly between large or small males (Table 1). Females accepted more attempted copulations than expected for both large and small males (Fig. 1c). Most attempted copulations resulted in mating, and few (<5%) females rejected mates (Fig. 1c).

Females mated with large or small males with equal frequency (Fig. 4a). The size of RHTLs of mated or unmated males (Fig. 4b), and the mean RHTL differences between mated and unmated pairs were not significantly different (Fig. 4c). The mean copulation times of large ( $20.2 \pm 3.1$ ) and small males ( $18.8 \pm 2.4$  s) were not significantly different ( $Z=0.49$ ,  $N_1=15$ ,  $N_2=8$ ,  $P=0.63$ ), nor were the copulation latencies (large males,  $128.0 \pm 58.4$ ; small males,  $69.2 \pm 20.4$  s; large sample Mann–Whitney  $U$ ,  $Z=0.21$ ,  $N_1=15$ ,  $N_2=9$ ,  $P=0.84$ ).

### Mate Choice by *C. flavipes* Males

Males first approached large or small females with similar frequencies, but more frequently attempted copulation first with small females (Table 1). The latencies of precopulatory behaviors towards large or small females did not differ significantly (Table 1). First attempted copulations were accepted more frequently by small females than by large females (Fig. 1d).

Males mated significantly more frequently with small females than with large females (Fig. 5a), and mated females were significantly smaller than unmated females (Fig. 5b). The mean difference in RHTLs between mated females and males was larger than the mean RHTL difference between unmated females and males (Fig. 5c).

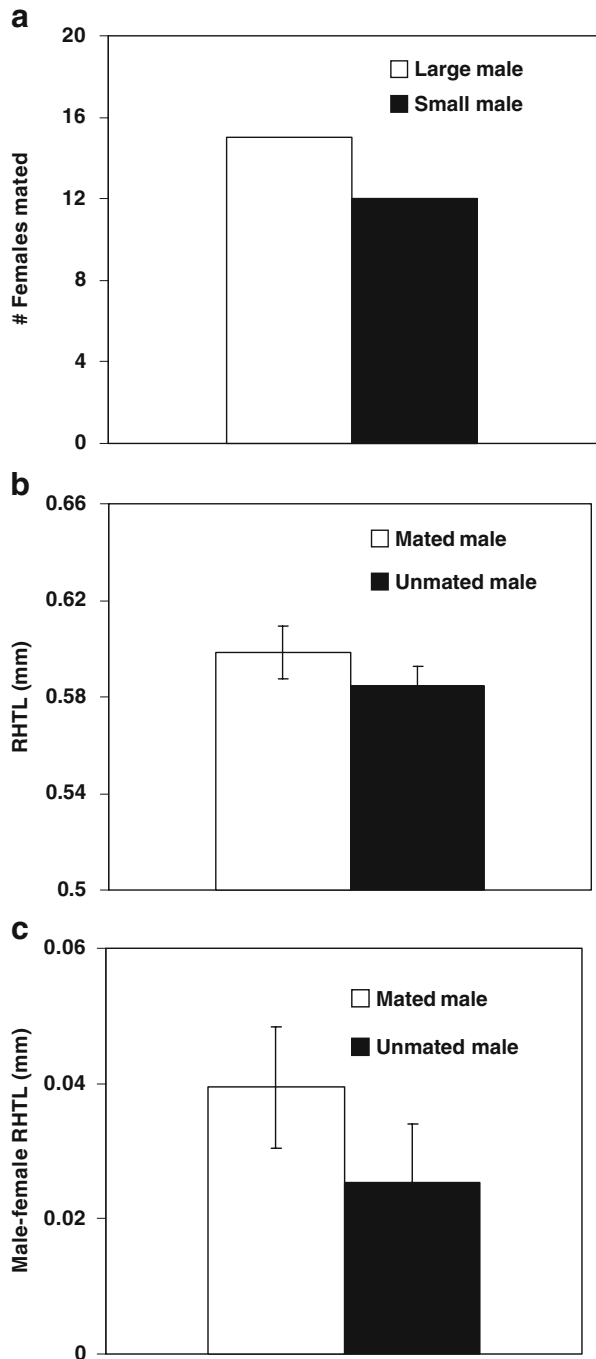
The mean copulation times in the female and male choice experiments of *C. flavipes* were not significantly different (female choice,  $19.8 \pm 2.8$ ; male choice,  $17.1 \pm 1.0$  s; large sample Mann–Whitney  $U$ ,  $Z=1.10$ ,  $N_1=24$ ,  $N_2=20$ ,  $P=0.27$ ), nor were the copulation latencies (female choice,  $116.3 \pm 34.4$ ; male choice,  $99.6 \pm 23.4$  s; large sample Mann–Whitney  $U$ ,  $Z=0.01$ ,  $N_1=27$ ,  $N_2=20$ ,  $P=0.99$ ).

## Discussion

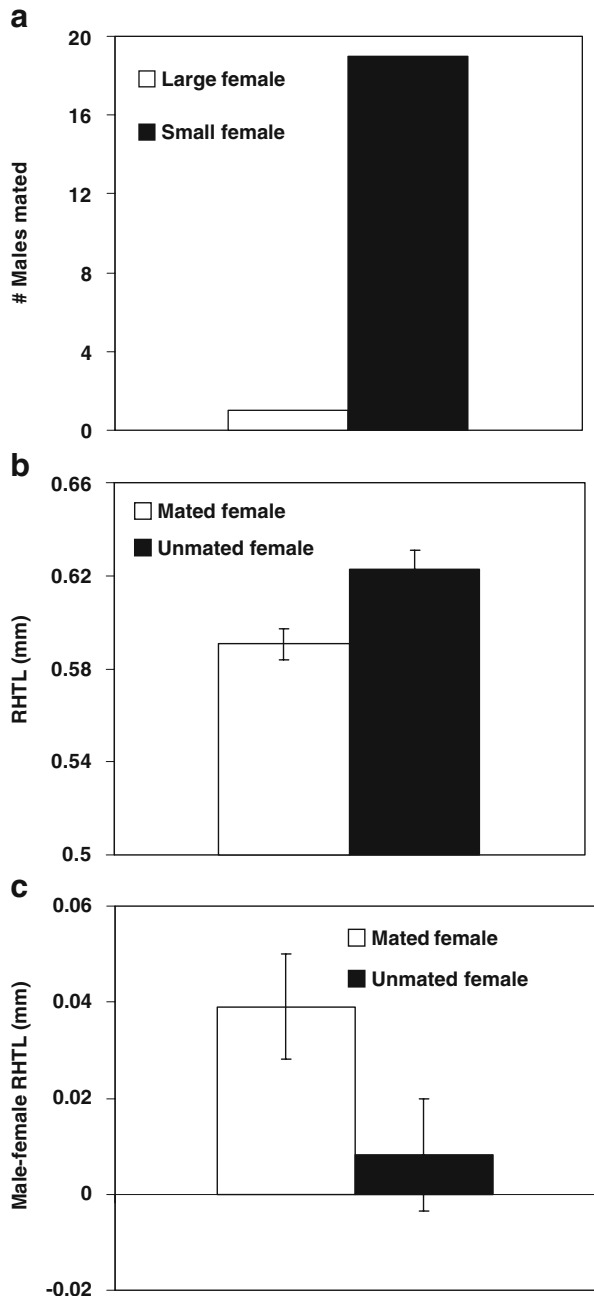
Male precopulatory behaviors in the *C. marginiventris* female choice experiment suggested a competitive advantage for large males, because large males approached females first more frequently than small males (Table 1). A large-male advantage has been demonstrated for solitary parasitoids other than *C. marginiventris*. Eggleton (1990) found that large males of the solitary *Lytarmes maculipennis* (Kamath and Gupta) (Ichneumonidae) mated more frequently than small males. Thus, once *C. marginiventris* males are in the vicinity of females, large males may have a competitive advantage over small males that may enhance their reproductive success.

Female choice for larger male mates may occur by active female choice, or it may result from passive choice, where females mate with the winner of a male competition

**Fig. 4** Mate choice for large or small males by *C. flavipes* females. **a** Females mated with large or small males with similar frequency (Chi-square test:  $\chi^2_1 = 0.41$ ,  $P=0.41$ ). **b** Mated and unmated males were similar in size (*t*-test:  $t_{48}=1.02$ ,  $P=0.31$ ). **c** The RHTL size difference between mated males and females was not significantly different compared to the RHTL difference of unmated males and females (paired *t*-test:  $t_{26}=1.16$ ,  $P=0.26$ ).



**Fig. 5** Mate choice for large or small females by *C. flavipes* males. **a** Males mated more frequently with small females (Chi-square test:  $\chi^2_1 = 28.90$ ,  $P < 0.001$ ). **b** Mated females were significantly smaller than unmated females ( $t$ -test:  $t_{38} = 3.16$ ,  $P < 0.001$ ). **c** The RHTL size difference between males and mated females was significantly different compared to males and unmated females (paired  $t$ -test:  $t_{20} = 4.68$ ,  $P < 0.001$ ), with a larger size difference between males and mated females.



(Parri et al. 1998; Blanchenhorn et al. 2000). It can be difficult to separate male competitive ability from female choice. Large males of *C. marginiventris* approached females first more frequently than small males, suggesting that females mate with the larger male by passive female choice. *C. marginiventris* females mated more

frequently with large males compared to small males (Fig. 2a–c), but larger males may be physically stronger so more able to copulate with females than smaller males. However, females more frequently rejected copulation attempts by small males, and accepted copulation attempts by larger males (Fig. 1a), suggesting that females actively choose to copulate with large males.

*C. marginiventris* males are not known to offer females any direct benefits, such as parental care of offspring, or nutritional resources that would increase female survival or longevity (Godfray 1994). It is possible that female *C. marginiventris*, which presumably mate only once, mate with large male mates to receive indirect benefits, such as larger offspring that could live longer, disperse farther, or produce offspring with a greater competitive ability to acquire mates. Female size preferences for mates have been documented where there is no apparent direct benefit of mate choice. For example, females of the green stink bug *Acrosternum hilare* (Say) (Pentatomidae) chose larger males as mating partners (Capone 1995), though there is no apparent advantage of mating with larger males. Male and female parasitoid size may be heritable (Ellers et al. 2001), though it can be influenced by factors such as host size (Charnov et al. 1981; Joyce et al. 2002; Cheng et al. 2003), including in *C. marginiventris* (Ramírez-Romero et al. 2007). Larger females are known to disperse farther and search larger areas for hosts (Ellers et al. 1998), and such size-related advantages may extend to males. Mating success for males can be limited by their ability to find females (van den Assem et al. 1989). Larger male parasitoids typically live longer than smaller males, and may have higher fitness than small males (van den Assem et al. 1989; Kazmer and Luck 1995; Bernal et al. 2001; Sagarra et al. 2001). Thus, females mating with larger males could receive an indirect benefit such as male offspring with greater competitive ability.

Females of the gregarious parasitoid, *C. flavipes*, mated equally with large or small males in the female choice experiment (Fig. 4a–b). *C. flavipes* produces broods that typically consist of 80% females, i.e. ~1♂:4♀♀ (Wiedenmann et al. 1992). Thus, males could be a limited resource, which could preclude females from being selective about mate size. On average males were larger than females in this experiment (Fig. 4c), which may explain in part why nearly all attempted copulations by males resulted in matings (Fig. 1c). *C. flavipes* is a partially local mating species (Bernal, unpublished data), and females may be less selective for mates if they are mating with brothers having similar genetic backgrounds (Hardy 1994). In addition, complementary sex determination does not occur in *C. flavipes* (Niyibigira et al. 2004), so mating among brothers and sisters would not lead to diploid males and reduced reproductive success (Godfray 1994; Ode et al. 1995; de Boer et al. 2007).

Other mate choice studies of gregarious parasitoids have examined mate choice for siblings or nonsiblings, and the level of male competition. Females of *C. glomerata* (L.) (Braconidae), a gregarious parasitoid with a female-biased sex ratio, showed no mating preference between siblings and non-siblings, although the trend was to mate with non-siblings (Gu and Dorn 2003). In *C. glomerata*, 30% of males and 50% of females dispersed before mating, and some male fighting for females was observed in natal patches where there were large numbers of males. Another study of *C. glomerata* found that male size did not influence mating success (Tagawa 2002). Ode et al. (1995) found that females of the gregarious parasitoid

*B. hebetor* Say (Braconidae) were more likely to mate with non-siblings than siblings, possibly because inbreeding produces diploid males with reduced viability. *Melittobia australica* Girault (Eulophidae), a gregarious species, has brachypterous males that mate at the emergence site. Males of this species are equally combative with siblings and non-siblings, suggesting no chemical recognition of siblings (Abe et al. 2005). In contrast, several other studies of gregarious parasitoids did not suggest a large-male precedence or advantage in mating (Suzuki and Hiehata 1985; Cheng et al. 2003). For gregarious parasitoids such as *C. flavipes*, chemical cues and recognition of siblings may be more important than size in relation to mate choice.

A conspicuous aspect of male courtship in *Cotesia* species is their wing fanning behavior (Sivinski and Webb 1989; Field and Keller 1993a; Kimani and Overholt 1995; Joyce et al. 2008), which produces low amplitude sound and substrate vibrations detected by females. In a study by van den Assem and Putters (1980), older male parasitoids prevented from wing fanning were less successful in mating than young males prevented from wing fanning. However, replay of courtship sounds and vibrations improved mating success for the older males, indicating that courtship sounds/vibrations convey information about male quality (van den Assem and Putters 1980). In other arthropods, such as the wolf spider *Hygrolycosa rubrofasciata* (Ohlert), males drum their abdomens on leaves to attract females. Females choose males that drum loudest, which correlated with male viability rather than male size (Kotiaho et al. 1996; Mappes et al. 1996). Future studies might investigate whether larger male *C. marginiventris* or *C. flavipes* produce louder courtship vibrations than smaller males, indicating male quality or good genes (Greenfield 2002), and whether they induce receptivity in females more quickly than smaller males.

In conclusion, mate choice in the solitary parasitoid *C. marginiventris* may occur by either passive or active female choice, although in this study it was difficult to separate the two. Mate choice may be more likely for solitary, outbreeding parasitoids such as *C. marginiventris* than for gregarious, inbreeding parasitoids such as *C. flavipes*, which mate siblings sharing genetic backgrounds. In partially local mating species such as *C. flavipes*, competition for mates at the emergence site may be moderated by dispersal and outbreeding.

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