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Family planning in a stemborer parasitoid: sex ratio, brood size and size-fitness relationships in *Parallorhogas pyralophagus* (Hymenoptera: Braconidae), and implications for biological control

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Abstract

Various aspects were studied of the brood size and sex allocation strategies, and of size-fitness relationships in *Parallorhogas pyralophagus* (Marsh), a gregarious ectoparasitoid of *Eoreuma loftini* Dyar. Brood size was significantly correlated with host size; larger hosts were allocated larger broods. Brood sex ratios were fixed precisely at 1 male per 4 females, and eggs were likely to be deposited in that order; differential mortality did not contribute to this precise sex ratio. The sex allocation strategy of *P. pyralophagus* is likely to conform to strict, i.e. single foundress, local mate competition. Adoption of this strategy is probably influenced by a limited insemination capacity of males; a smaller proportion of females (0.09 vs. 0.21) remained virgin in broods with precise or higher sex ratios (≥ 0.20 males) relative to broods with lower than precise sex ratios (< 0.20 males). Moreover, all females were inseminated in most broods (60%) with precise or higher sex ratios, whereas this did not occur in broods with lower than precise sex ratios. The hypothesized occurrence of strict local mate competition in *P. pyralophagus* was supported also by observations that: (i) offspring brood sex ratios were independent of maternal brood sex ratios and number of parental females concurrently allocating offspring to a group of hosts, and; (ii) the rate of superparasitism under no-choice conditions was low (~20%), suggesting that rates of outbreeding in the field are low. Other results suggested that fitness in *P. pyralophagus* was correlated with adult size; longevity and reproductive capacity both increased with adult size in males and females. However, adult size may be more important for females than for males because the differences in reproductive capacity between the largest and smallest individuals was up to 7.3 times greater in females versus < 2 times in males.

Introduction

Parallorhogas pyralophagus (Marsh) (Hymenoptera: Braconidae) is a gregarious external parasitoid of gramineous stemborers that was discovered in Mexico and imported to Texas, USA, in 1981 for biological control of Mexican rice borer, *Eoreuma loftini* Dyar (Lepidoptera: Pyralidae) (Smith *et al.*, 1987). *Eoreuma loftini* is the key pest of sugarcane in the Rio Grande Valley of Texas (Meagher *et al.*, 1998), and an important pest of maize in southern Texas and north-eastern Mexico (Rodríguez del Bosque *et al.*, 1988; Youm *et al.*, 1988). *Parallorhogas pyralophagus* also parasitizes the sugarcane borer *Diatraea saccharalis* Fabricius (Lepidoptera: Pyralidae), another important pest of

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sugarcane in south Texas and elsewhere, but field parasitism rates are typically low (Dasrat *et al.*, 1997; Meagher *et al.*, 1998). Other hosts of *P. pyralophagus* include various species of *Diatraea, Tryporyza* and *Chilo* (Smith *et al.*, 1987).

Laboratory studies conducted following importation of *P. pyralophagus* documented its biology and life history (Melton & Browning, 1986; Smith *et al.*, 1987). Among other biological characteristics, these studies showed that *P. pyralophagus* is an idiobiont parasitoid; females paralyse their hosts, which are concealed in their feeding tunnels within plant stems, prior to oviposition. Moreover, *P. pyralophagus* reproduces by arrhenotokous parthenogenesis and sex determination is haplo-diploid; females are hence able to determine the sex of offspring through selective fertilization of eggs. Thus, *P. pyralophagus* females could gauge host quality (size) at the time of oviposition, and allocate optimal numbers of male and female offspring to each host. Early observations suggested that *P. pyralophagus* females adjusted brood size (number of parasitoid offspring allocated per host) according to host size, and that sex ratios were strongly female-biased (Melton & Browning, 1986; Smith *et al.*, 1987; personal observations).

Female-biased sex ratios are frequently displayed by gregarious parasitoids as matings predominantly occur between siblings in a brood and males compete against one another for mates; this phenomenon is referred to as local mate competition (Hamilton, 1967; Godfray, 1994). Offspring sex ratio optima under local mate competition are expected to vary according to the number of parental females contributing to a group of interbreeding offspring and the prevalence of matings between individuals from different groups (Hamilton, 1967; Godfray, 1994; Hardy *et al.*, 1998). If a single female produces each offspring group and matings between groups are rare, i.e. local mate competition is strict, then it is expected that each offspring group should include only enough males to inseminate all females. If more than one female contributes to an offspring group, then the proportion of male offspring increases asymptotically until it equals that of female offspring (Hamilton, 1967; Godfray, 1994; Hardy *et al.*, 1998). Because *P. pyralophagus* is a gregarious parasitoid and its hosts typically do not occur in dense aggregations in agricultural crops (Meagher *et al.*, 1996; personal observations), it was expected that in the field offspring groups would be produced by individual females and that matings would occur largely among individuals from a single brood. Thus, it was expected that local mate competition would be strong in *P. pyralophagus*. However, local mate competition is thought to be constrained in the Ichneumonoidea, including Braconidae, by single-locus complementary sex determination (Butcher *et al.*, 2000a,b).

In the light of these observations, the studies reported herein addressed whether *P. pyralophagus* females adjust the sex and number of offspring allocated per host based on host size. In addition, because *P. pyralophagus* is a gregarious parasitoid, the possibility was examined that female *P. pyralophagus* adjust their offspring sex ratio (proportion males) in response to local mate competition (Hamilton, 1967). Moreover, the sex ratios of individual *P. pyralophagus* broods were analysed to determine whether they displayed a 'precise' sex ratio pattern (*sensu* Green *et al.*, 1982), and whether parental females incur a fitness cost by producing broods with lower than the 'precise' sex ratio. Finally, in addition to examining the sex allocation strategy of *P. pyralophagus*, these studies addressed other aspects of its biology that had not been considered in previous studies such as: (i) the effects of adult size and food availability on female and male partial fecundity; (ii) the effects of adult size on female and male longevity; and (iii) whether females avoid superparasitizing their hosts.

Material and methods

Host and parasitoid cultures

All experiments were conducted using *E. loftini* as the host. Environmental conditions for rearing and for all experiments were set at 25 ± 1°C, 14L:10D photoperiod, and 50–70% relative humidity, except as noted. *Eoreuma loftini* and *P. pyralophagus* were reared following previously described methodology; rearing of the former was accomplished using artificial diet (Martinez *et al.*, 1988; Smith *et al.*, 1993).

Adult parasitoids were produced by placing individual *E. loftini* larvae, between 28 and 32 days old, inside 2 cm long waxed paper straw segments (6 mm diameter). The straws were plugged at both ends with artificial diet, and then sealed by dipping each end in melted paraffin wax. Single larvae inside straws were offered for 24 h to individual mated female parasitoids inside 12 × 35 mm glass shell vials plugged with cotton. After 24 h, the female was removed and the larva within the straw was incubated inside the shell vial. Emerging male–female parasitoid pairs were isolated inside 9 × 30 mm shell vials plugged with cotton and provided with honey. Females were used in experiments when they were 2–4 days old, except as noted.

Influence of host size and maternal brood sex ratio on offspring brood size and sex ratio

Individual female parasitoids were offered a single host larva inside a 2 cm straw for 24 h. For each female, the weight (mg) of each host larva, and the size and sex ratio of the maternal brood (i.e. the female's brood of origin) were recorded. Sex ratios were estimated as the proportion of males relative to total individuals. Maternal broods with atypically high sex ratios (i.e. weaker female bias) were obtained by adding adult males (2–4 days old) from other broods; males were added to maternal broods as soon as the first individual emerged, typically a male, and females were never removed or added for manipulating brood sex ratios. In general, all maternal broods with > 40% males used in these experiments were produced by this method, whereas none of the broods with < 30% males were manipulated. The number and sex ratio of adult parasitoids emerging from each straw were recorded. This procedure was repeated 188 times, and yielded 142 broods. The resulting data were used to explore relationships between: (i) host size and brood size; (ii) maternal brood sex ratio and offspring brood sex ratio; and (iii) offspring brood size and sex ratio. Potential relationships between these variables were explored via linear regression analyses (Zar, 1996). Offspring broods of < 5 wasps (4/142 broods) were excluded from the analyses because sex ratios were found to conform to a pattern of 1 male:4 females (see Results). All sex ratio estimates were transformed to their arcsine values, with Anscombe's transformation, prior to analyses (Zar, 1996). Unless indicated otherwise, all proportions and rates are presented followed by their standard deviation, and means followed by their standard error.

A second experiment assessed the relationship between host size and brood (clutch) size based on numbers of parasitoid eggs allocated per host. The protocol was as described above, except that the straws containing the host were cut open immediately after exposure to females and the number of *P. pyralophagus* eggs within was recorded. This procedure was repeated 110 times and yielded 75 broods. The potential relationship between host size and brood size, based on *P. pyralophagus* eggs, was examined via linear regression analysis (Zar, 1996).

Precise sex ratios

The precision of *P. pyralophagus* brood sex ratios was determined in two ways. Firstly, the mean sex ratio from 142 individual broods produced by isolated females (see above) was tested for consistency with a hypothesized sex ratio of precisely 1 male per 4 females (= 0.20) via a log-likelihood goodness of fit test (Zar, 1996). Secondly, the expected numbers of males in broods of 5–20 wasps were calculated under two assumptions: male eggs laid first, or male eggs laid last, in a sequence of five eggs. The binomial test (Green *et al.*, 1982) was then used to examine whether the number of broods observed with exactly the expected number of males was significantly greater than predicted if brood sex ratios were binomially distributed; i.e. the test provides a conservative estimate of the probability of observing as many as k (or more) broods with exactly the predicted number of males. This probability was estimated as:

$$\alpha = \sum_{x=k}^{n} \binom{n}{x} \hat{P}^{x} \left(1-\hat{P}\right)^{n-x}$$

where k is a random variable with a binomial distribution, with parameters n and P, when P is no larger than P. The parameter P is the maximum possible probability of having exactly the expected number of males per brood (1 male per 4 females in this study). P was calculated as

$$\hat{P} = \left(1-1/N\right)^{N-1}$$

where N = brood size (Green *et al.*, 1982).

Influence of the number of parental females exploiting a common group of hosts on offspring sex ratios

Groups of 1, 4 or 8 newly emerged females and a corresponding number of males were held for two days inside 15 × 45 mm shell vials with honey; each of the females in each group was obtained from a different brood and thus were not siblings. After two days, the males were removed, and the females (i.e. foundresses) were transferred to a covered arena where they were offered eight straw segments (2.5 cm length), each containing one host larva (35–65 mg), for 24 h. The arena consisted of two Petri dish bottoms (140 mm diameter × 17 mm deep) placed one atop the other, facing inward, and held together with rubber bands. The straw segments were secured in an upright position, in an evenly spaced circular distribution in the arena, by pressing the still-warm waxed end of the straw against the bottom of the arena. After 24 h, the females were removed from the arena and the straws were placed inside individual 12 × 35 mm shell vials plugged with cotton. The numbers of parasitoids emerging per host were counted and their sex determined. Six trials involving one foundress, six trials involving four foundresses, and four trials involving eight foundresses were conducted; trials not yielding adult parasitoids or with broods consisting solely of males were excluded from the analyses. The sex ratios corresponding to each trial were arcsine transformed and means were compared via single factor analysis of variance (Zar, 1996).

Differential pre-imaginal mortality and brood sex ratio

Clear gelatin capsules (size 0) containing individual hosts were exposed to individual female parasitoids for 24 h inside 24 × 35 mm glass shell vials plugged with cotton. The gelatin capsules were examined under a stereomicroscope after 24 h, and the number of *P. pyralophagus* eggs within was recorded. The hosts inside the gelatin capsules were incubated, and the number and sex of parasitoids emerging from each host were recorded and compared against the corresponding number of eggs. Broods in which all eggs successfully developed into adults were combined into one group (no pre-imaginal mortality), and broods in which at least one egg failed to develop into an adult were combined into a second group (some pre-imaginal mortality). Sex ratios of broods with no and some pre-imaginal mortality were compared via a log-likelihood contingency table test (Zar, 1996).

Superparasitism

Individual (first) females were offered a single larva (35–65 mg) inside a 2 cm straw for 24 h. The female was removed after 24 h and the larva was examined for signs of parasitism; parasitized larvae are completely paralysed or lethargic. All parasitoid eggs were counted and removed from paralysed host larvae with a fine paintbrush. Paralysed larvae were transferred to new 2 cm straws and offered to a different (second) female for 24 h. After 24 h, each larva was again inspected for *P. pyralophagus* eggs to determine whether it had been parasitized by the second female, and the number of eggs was recorded. It was hypothesized that females do not discriminate between paralysed and healthy hosts, and thus all paralysed larvae would be superparasitized by second females. The observed superparasitism rate was compared against the expected rate, i.e. 100%, via a log-likelihood goodness of fit test (Zar, 1996). The mean and variance of the second female's brood size were compared against those of the first female using a paired-sample *t*-test and Bartlett's test for homogeneity of variances, respectively (Zar, 1996).

Female virginity in relation to brood sex ratio

The proportion of virgin females in broods with precise or higher sex ratios, i.e. ≥ 1 male per 4 females, was compared against that in broods with lower than precise sex ratios, i.e. < 1 male per 4 females. Individual females were offered a single host larva inside a 3 cm straw segment for 24 h. Sixteen days later, each straw was transferred to an emergence chamber designed to discriminate females that were inseminated by a common-brood male sibling prior to dispersing from the emergence site (straw), from females that dispersed uninseminated. Straws were placed upright inside a 12 × 35 mm shell vial secured to the bottom of a Fisher™ tri-cornered polypropylene beaker (400 ml, 80 mm upper diameter). An inverted Pyrex™ 60° angle funnel

(75 mm diameter, 75 mm stem) was fastened to the beaker with poster putty and a 25 × 95 mm shell vial placed over the funnel's stem to collect dispersing parasitoids. The emergence chambers were maintained at room temperature (21–23°C) under artificial lighting between 08:00 h and 17:00 h, and monitored at intervals < 15 min to collect dispersing parasitoids. Dispersing females were immediately dissected in a droplet of physiological saline to determine whether their spermathecae contained sperm, an indication that they had been inseminated prior to dispersing. Trials were terminated after seven days if no parasitoids or only males remained within the chamber, and broods in which a female coincided with a male inside the collection vial were discarded. The sex ratios of six broods were manipulated by removing the first-emerged male; males were removed only if they emerged prior to any females. This was necessary because *P. pyralophagus* broods with sex ratios < 0.20 are uncommon. Proportions of virgin females were estimated separately for broods with sex ratios ≥ 0.20 and those with sex ratios < 0.20, and were compared via a log-likelihood contingency table test (Zar, 1996).

Adult parasitoid size versus egg load, insemination capacity and longevity

Potential relationships between parasitoid adult size and egg load, insemination capacity and longevity were explored using the length of the hind tibia as an index of adult size. An adult size–egg load relationship was assessed for female parasitoids of different ages in the presence and absence of food: (i) newly emerged (< 24-h-old); (ii) 4-day-old, either provided with or deprived of honey; and (iii) 7-day-old either provided with or deprived of honey. Females were dissected in a droplet of 70% ethyl alcohol and the number of ovarian eggs was counted. An adult size-insemination capacity relationship was assessed in male parasitoids using the emergence chambers and protocol described previously. In this case, only broods with single males and lower than precise sex ratios (< 0.20) were included in the analyses. Thirty-four trials were conducted, which yielded 13 broods suitable for analysis. The proportion of inseminated females in a brood was determined through dissection in physiological saline. Adult parasitoid size–longevity relationships in the absence of food were examined for females and males. Newly emerged parasitoids of each sex were individually isolated in shell vials (9 × 30 mm) plugged with cotton, and inspected daily until their death. Relationships between adult size and egg load, insemination capacity (arcsine transformed proportions), and longevity were explored via linear regression analyses; *t*-tests were used to compare the rates of gain in these fitness parameters with adult size between females and males (Zar, 1996).

Results

Influence of host size and maternal brood sex ratio on offspring brood size and sex ratio

Parasitoid brood size was significantly influenced by host size (fig. 1). Larger hosts yielded larger broods both based on the number of adult parasitoids emerging per host (fig. 1a) and number of eggs allocated per host (fig. 1b). Adult brood size appeared greater than egg clutch size but their regression slopes were not significantly different ($P = 0.50$, $t = 1.97$, 213 df). Offspring brood sex ratios were not influenced either by offspring brood sizes (fig. 2a) or maternal brood sex ratios (fig. 2b).

Precise sex ratios

Individual brood sex ratios (pooled sex ratio = 0.196 ± 0.010, n = 1506) were not significantly different from a hypothesized sex ratio of 0.20, i.e. 1 male:4 females ($P > 0.999$, $G = 13.47$, 141 df). Broods with exactly the predicted number of males occurred more often than expected under the assumption of binomial variance for several brood sizes (table 1); brood size frequencies were small or nil for broods in which the predicted number of males did not deviate significantly from expected. Precise sex ratios were evident both when the male egg was presumed to be deposited first (table 1a) or last (table 1b) in a sequence of five eggs. However, the precise sex ratio pattern appeared to be more prevalent in the case where male eggs were presumed to be deposited first (9/10 broods where $n \geq 4$) versus last (7/10 broods where $n \geq 4$) in a sequence of five eggs.

Influence of the number of parental females exploiting a common group of hosts on offspring sex ratios

Single females and groups of females produced offspring with similar sex ratios (fig. 3). The trend toward increased sex ratio with number of parental females was not significant ($P = 0.15$)

Differential pre-imaginal mortality and brood sex ratio

Thirty-four of 40 hosts offered to individual females were parasitized, nine broods suffered no pre-imaginal mortality, and 25 suffered some pre-imaginal mortality. The sex ratio of broods suffering no pre-imaginal mortality (0.14 ± 0.03) was not significantly different from that of broods suffering some pre-imaginal mortality (0.18 ± 0.03) ($P = 0.49$, $G = 0.813$, 1 df). This indicated that the observed sex ratio patterns were not influenced by differences in pre-imaginal mortality rates between males and females.

Superparasitism

One hundred and six of the 149 larvae exposed to parasitism by first females were successfully parasitized and thus subject to superparasitism. Most females avoided parasitizing previously paralysed hosts; only 19.8% ± 0.04% of second females offered parasitized hosts superparasitized these hosts. This rate was lower than expected under the hypothesis that females do not discriminate previously parasitized hosts ($P < 0.001$, $G = 172.25$, 1 df). The mean number of eggs allocated to superparasitized hosts by second females (5.2 ± 1.1) did not differ from that allocated by first females (6.7 ± 0.6) ($P = 0.267$, $t = 1.141$, 20 df). However, greater variance was associated with second-female broods ($s^2 = 23.89$) relative to first-female broods ($s^2 = 7.21$) ($P = 0.010$, $F = 0.302$, 40 df).

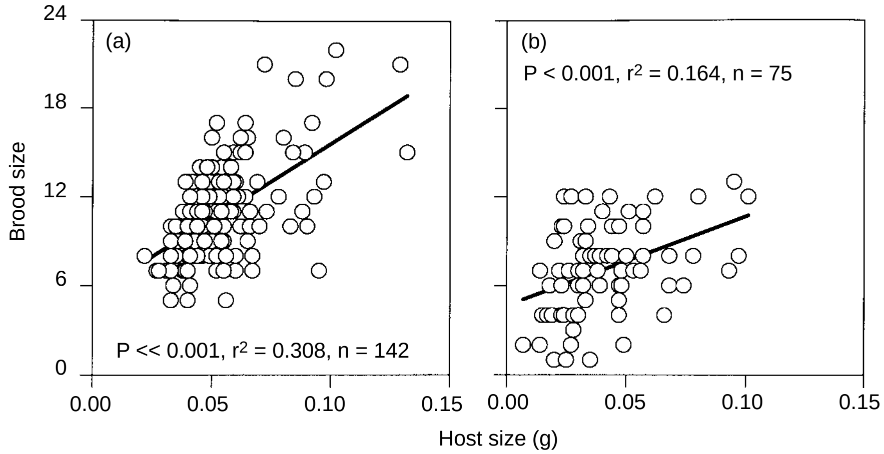

Fig. 1. The influence of *Eoreuma loftini* host size on *Parallorhogas pyralophagus* brood size based on (a) the number of *P. pyralophagus* adults emerging per host (y = 5.40 + 101.84x), and (b) the number of *P. pyralophagus* eggs allocated per host (y = 4.65 + 60.30x).

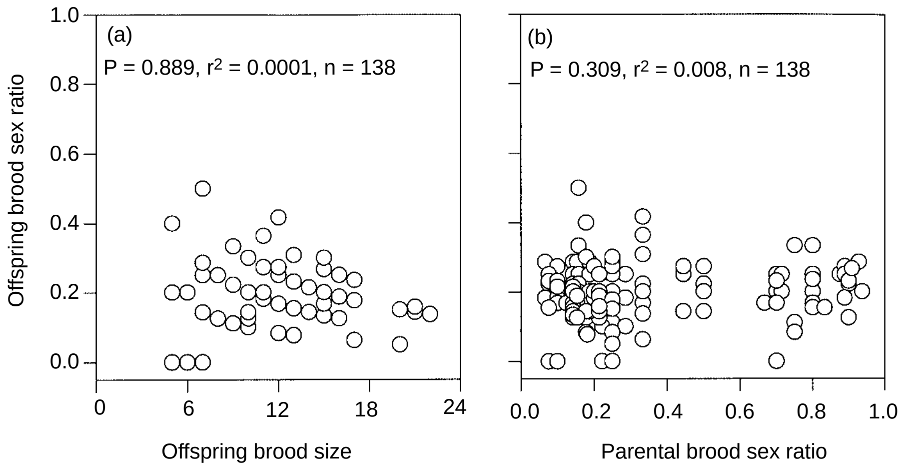

Fig. 2. (a) The sex ratio (proportion males) of *Parallorhogas pyralophagus* broods in relation to brood size (y = 34.13 + 0.06x). (b) The sex ratio of *P. pyralophagus* broods in relation to the sex ratio of the maternal brood (i.e. the parental female's brood of origin) (y = 25.19 + 0.03x). Arcsine-transformed sex ratio values were used for analyses, and untransformed values are shown in both (a) and (b).

Female virginity in relation to brood sex ratio

Twenty-five of 64 trials yielded broods with sex ratios ≥ 0.20 and 15 with sex ratios < 0.20, while 24 were discarded due to coincidence of males and females in the collection vial. A smaller proportion of females remained virgin in broods with precise or higher sex ratios (sex ratio ≥ 0.20) relative to broods with lower than precise sex ratios (sex ratio < 0.20) (fig. 4). Moreover, in none of the broods with lower than precise sex ratios were all females successfully inseminated, whereas all females were successfully inseminated in most (60%) broods with precise or higher sex ratios. In addition, there was no consistent relationship between virginity and brood size ($P = 0.09$, $r^2 = 0.07$, $b = 1.14 \pm 0.66$, df = 1, 39).

Adult parasitoid size versus egg load, insemination capacity and longevity

Large females had greater egg loads than smaller females independent of age and access to food (fig. 5). Egg load appeared to increase at a greater rate with female size for older females, but to increase at similar rates with adult size for females provided food relative to those denied food. Regression estimates showed that the largest females had egg loads between 3.8 times (4- and 7-day-old old females without honey) and 7.3 times (7-day-old females with honey) greater than those of the smallest females.

Large males inseminated a greater proportion of their common-brood siblings relative to smaller males (fig. 6). The

Table 1. Distribution of *Parallorhogas pyralophagus* brood sizes, and ∝, the upper bound of the probability of observing *k* or more broods with the expected number of males under a binomial distribution of sex ratios.

Brood size (N)	Frequency (n)	Expected no. of males	No. with expected no. of males (*k*)	∝
(a) The male egg is deposited first in a sequence of five eggs				
5	3	1	1	1.000
6	1	2	0	1.000
7	12	2	7	0.003
8	18	2	13	7×10^{-7}
9	15	2	9	3×10^{-4}
10	15	2	11	2×10^{-6}
11	13	3	5	0.002
12	15	3	4	0.023
13	12	3	4	0.009
14	6	3	2	0.062
15	7	3	5	3×10^{-5}
16	4	4	1	0.121
17	2	4	1	0.060
18	0	4	0	1.000
19	0	4	0	1.000
20	2	4	0	1.000
(b) The male egg is deposited last in a sequence of five eggs				
5	3	1	1	0.794
6	1	1	0	1.000
7	12	1	2	1.000
8	18	1	5	1.000
9	15	1	3	1.000
10	15	2	11	2×10^{-6}
11	13	2	7	0.002
12	15	2	7	0.006
13	12	2	5	0.030
14	6	2	4	0.007
15	7	3	5	3×10^{-5}
16	4	3	2	0.025
17	2	3	1	0.128
18	0	3	0	1.000
19	0	3	0	1.000
20	2	4	0	1.000

largest males used in these trials were ~1.5 times more successful than the smallest males.

Large parasitoids, both female and male, lived longer than smaller ones (fig. 7). The largest females lived ~2.7 times longer than the smallest ones, while the largest males lived ~2.4 times longer than the smallest ones. Longevity increased with adult size at similar rates in females and males.

Discussion

Sex allocation strategy of P. pyralophagus

Overall, the results of this study suggest that female *P. pyralophagus*: (i) allocate more offspring to larger hosts relative to smaller ones; (ii) deposit precisely one male egg per every four female eggs; (iii) are likely to deposit male eggs first in each sequence of five eggs; and (iv) that this ratio and sequence of male and female eggs ensures that most to all daughters are inseminated by their common-brood siblings soon after emergence and prior to dispersing. In addition, *P. pyralophagus* females tended to avoid attacking previously paralysed hosts, which suggests that superparasitism is likely to be infrequent. Although superparasitism was not entirely absent, the observed rate (~20%) can be considered low given that females in the experiment were both inexperienced and exclusively offered previously parasitized hosts, factors known to increase the incidence of superparasitism (Godfray, 1994). In the field, avoidance of superparasitism will reduce the likelihood of outbreeding in a gregarious species such as *P. pyralophagus*.

The observed lack of influences of maternal brood sex ratio and increasing foundress number on offspring sex ratios, together with the above results suggest that the sex ratio strategy of *P. pyralophagus* conforms to a strict (i.e. single foundress) local mate competition strategy (Hamilton, 1967; Hartl, 1971; Godfray, 1994; Hardy *et al*., 1998). Under this hypothesis, most matings in *P. pyralophagus* would be expected to occur between wasps belonging to a single brood. An apparent limitation of the insemination capacity of males may be an important consideration for sex allocation in *P. pyralophagus* as exactly enough sons to inseminate all daughters should be allocated to each brood, leading to a constant and precise sex ratio independent of maternal brood sex ratio, foundress number and brood size. This contrasts with some other studies on gregarious parasitoids, which show that sex ratios decrease with brood size (e.g. Green *et al*., 1982; Hardy *et al*., 1998). In addition,

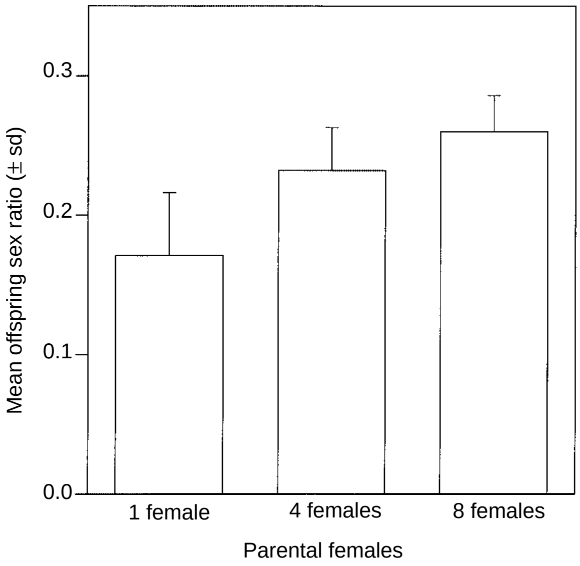

Fig. 3. The sex ratio (proportion males) of *Parallorhogas pyralophagus* offspring groups in relation to the number of parental females contributing offspring to each offspring group ($P = 0.148$, $F_{2, 13} = 2.22$).

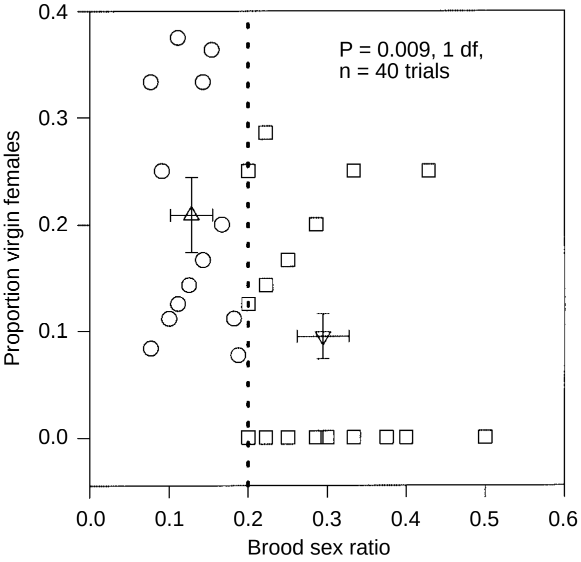

Fig. 4. The proportion of *Parallorhogas pyralophagus* virgin females in broods with a lower than precise sex ratio, i.e. < 0.20 males (circles, left of broken line), versus broods with a precise or higher sex ratio, i.e. $\geqslant 0.20$ males (squares, right of broken line). Mean proportions (± sd) are indicated by upright and inverted triangles, respectively.

the results did not support the prediction that virginity and brood size are negatively correlated under strict local mate competition (Heimpel, 1994). In contrast, the results were consistent with the prediction that virginity is low in parasitoids in which strict local mate competition is a major component of the mating structure (Godfray & Hardy, 1993); only eight (small) broods (1–7 individuals) of 142 consisted solely of females, and ~3% of all females had zero opportunity to mate at the natal site (data not shown).

The results of this study indicating the occurrence of a precise sex ratio and strict local mate competition sex ratio strategy in *P. pyralophagus* are unique because sex ratio strategies in which levels of sib-mating are expected to be high are thought to be constrained in the Ichneumonoidea. Single-locus complementary sex determination is thought to be an ancestral condition in the Ichneumonoidea, and some studies strongly support this hypothesis for specific subfamilies (Butcher *et al.*, 2000a,b). Under single-locus complementary sex determination, intense sib-mating rapidly leads to strongly male-biased sex ratios. The results of this study indicate that single-locus complementary sex determination does not occur in *P. pyralophagus*; strict local mate competition as suggested to occur in this parasitoid leads to a precise and female-biased sex ratio. Moreover, the results support the notion that more data than currently available are required to confirm the hypothesis that single-locus complementary sex determination is the ancestral condition in the Ichneumonoidea (Butcher *et al.*, 2000a,b).

Adult size and fitness in P. pyralophagus

Parallorhogas pyralophagus adult size was positively correlated with two fitness parameters, reproductive capacity and longevity, in both males and females. The largest females had egg loads up to 7.3 times greater than the smallest ones, insemination capacity of the largest males was < 2 times greater than that of the smallest ones, and differences in longevity between the largest and smallest individuals were slightly greater for females (2.7 times) than for males (2.4 times). Overall, our results suggested that adult size may be more important for female than for male *P. pyralophagus*. These results are consistent with those obtained for *Heterospilus prosopidis* Viereck (Hymenoptera: Braconidae) and *Lariophagus distinguendus* Förster (Hymenoptera: Pteromalidae) (Jones, 1982; van den Assem *et al.*, 1989). However, the results of a field study with *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) showed that differences in reproductive success between large and small individuals were less for females than for males (Kazmer & Luck, 1995). The fitness parameters evaluated in this study were measured under laboratory conditions and prior studies show that parasitoid size-fitness relationships assessed under laboratory conditions may not closely reflect those relationships under field conditions (West *et al.*, 1996). Therefore, the relationships found in this study should be viewed with caution.

Implications for biological control

The results showed that sex allocation by *P. pyralophagus* females is precise, and that the apparent limited insemination capacity of males may contribute to the precision of the sex ratio. Moreover, the results suggested that the sex ratio strategy of *P. pyralophagus* conforms to strict local mate competition, characterized by intense sib-mating, and that very few opportunities may be available for outbreeding as low rates of superparasitism are expected in the field. Finally, the results showed that adult size is an important factor influencing the fitness of *P. pyralophagus*. These findings have significant implications for various aspects of biological control efforts involving *P. pyralophagus*

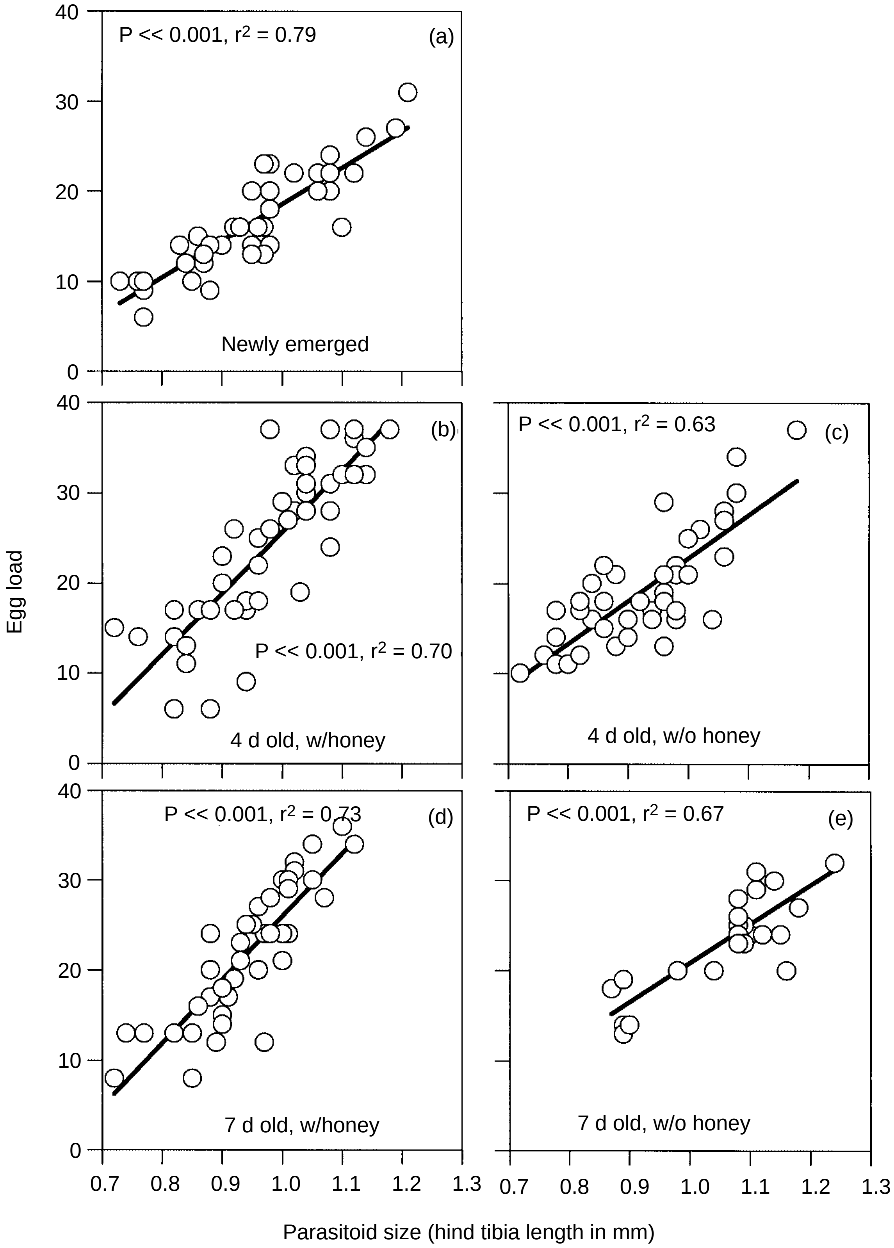

Fig. 5. The influence of adult size (length of hind tibia in mm) of female *Parallorhogas pyralophagus* on the egg load: (a) newly-emerged females (y = 40.62x – 22.07); (b) 4-day-old females provided with honey (y = 68.06x – 42.39); (c) 4-day-old females denied honey (y = 47.69x – 24.89); (d) 7-day-old females provided with honey (y = 70.37x – 44.39), and; (e) 7-day-old females denied honey (y = 43.30x – 22.43). The rates of gain in egg load are similar between (b) and (c) ($P > 0.500$, $t = 0.440$, 82 df) and between (d) and (e) ($P > 0.500$, $t = 0.436$, 64 df).

and other species with similar ecological characteristics. For example, some colonization efforts with imported parasitoids are believed to have failed due to Allee effects, low encounter rates between males and females and the resulting high virginity rates, when parasitoids are released in small numbers or too sparsely over extensive areas (Hopper & Roush, 1993). However, relative to other parasitoids (e.g. solitary parasitoids of isolated hosts), this may be less important for *P. pyralophagus* given its gregariousness, precise sex ratio and propensity for sib-mating. The propensity for sib-mating in *P. pyralophagus*, however, raises two other concerns, inbreeding depression and diploid males. Genetic loads and inbreeding depression are commonly thought to be low in species with haplo-diploid sex determination, such as *P. pyralophagus*. Although this may be the case for males, in which deleterious mutations may be rapidly eliminated due to their haploidy, it is less so for females. Diploid

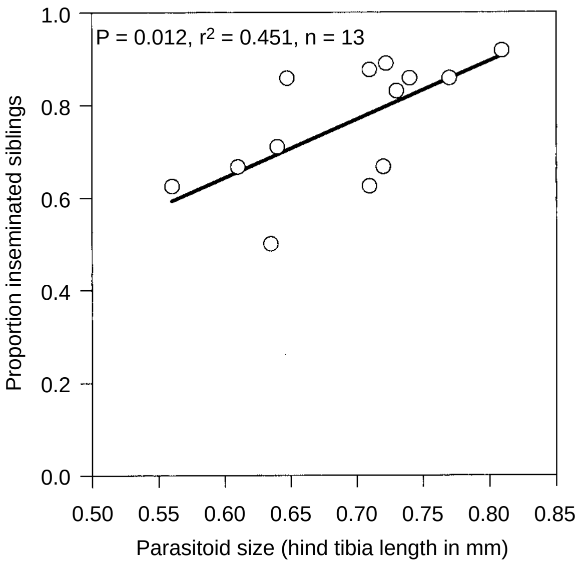

Fig. 6. The influence of adult size (length of hind tibia in mm) of male *Parallorhogas pyralophagus* on the proportion of common-brood female siblings that they inseminate in broods comprised of a single male and a variable number of females ($y = 1.28 + 86.83x$). Arcsine-transformed sex ratio values were used for analyses, and untransformed values are shown.

females under chronic inbreeding will have genetic loads, and thus will suffer inbreeding depression in female-only traits similar to those observed in diploid species (Werren, 1993; Antolin, 1999). Single-locus complementary sex determination, which is more prevalent in ichneumonoids relative to other parasitoid groups, may rapidly lead to strongly male-biased sex ratios and extinction in the laboratory in species where it is known to occur (Butcher *et al.*, 2000a,b). The results of this study, along with the lack of evident changes in the sex ratio over > 4 years (~18 generations per year) of continuous rearing in the laboratory (J.W. Smith, Jr., personal communication; personal observations), suggest that single-locus complementary sex determination does not occur in *P. pyralophagus*. Sex ratio manipulation to increase female parasitoid yields during rearing (e.g. Heinz, 1998; Bernal *et al.*, 1999) seems gratuitous and difficult with *P. pyralophagus*. In addition, any effects of inbreeding may be ameliorated by pooling individual broods produced by different females and concentrating their emergence in small cages to foster encounters between unrelated males and females. Finally, prior studies have shown that larger parasitoid females are more reproductively successful in the field than smaller females, and hence may be more effective augmentative biological control agents (Visser, 1994; Kazmer & Luck, 1995; West *et al.*, 1996; Bennett & Hoffman, 1998; Ellers *et al.*, 1998). The results of this study also suggested that larger *P. pyralophagus* females may be more reproductively successful than smaller females, but this remains to be verified in the field. However, opportunities for manipulating the size of *P. pyralophagus* females reared for field release were not exposed in this study. Improving the quality of gregarious parasitoids reared for field release by manipulating their adult size is a question that has received relatively little attention but that merits further investigation.

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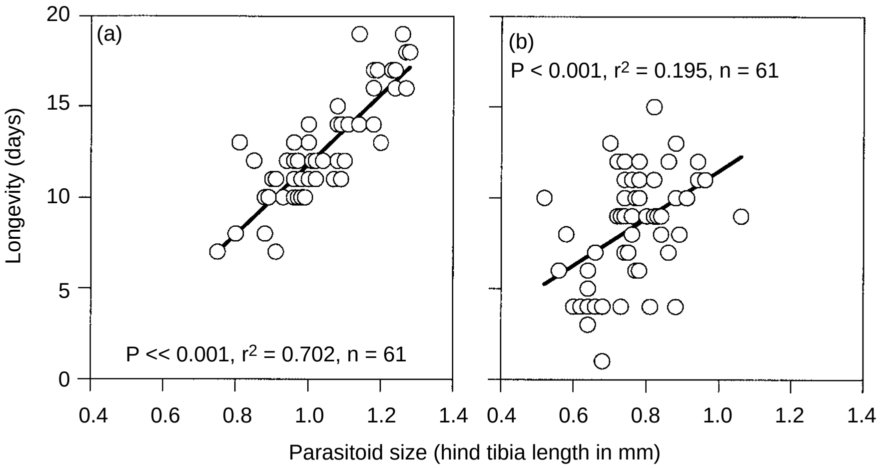

Fig. 7. The influence of adult size (length of hind tibia in mm) on longevity (days) in *Parallorhogas pyralophagus* (a) females ($y = 19.19x - 7.38$) and (b) males ($y = 13.02x - 1.52$). The rate of gain in longevity with adult size is similar between females and males ($P > 0.500$, $t = 0.351$, 118 df).

References

Antolin, M.F. (1999) A genetic perspective on mating systems and sex ratios of parasitoid wasps. *Researches in Population Ecology* **41**, 29–37.

Bennett, D.M. & Hoffmann, A.A. (1998) Effects of size and fluctuating asymmetry on field fitness of the parasitoid *Trichogramma carverae* (Hymenoptera: Trichogrammatidae). *Journal of Animal Ecology* **67**, 580–591.

Bernal, J.S., Luck, R.F. & Morse, J.G. (1999) Host influences on sex ratio, longevity, and egg load of two *Metaphycus* species parasitic on soft scales: implications for insectary rearing. *Entomologia Experimentalis et Applicata* **92**, 191–204.

Butcher, R.D.J., Whitfield, W.G.F. & Hubbard, S.F. (2000a) Single-locus complementary sex determination in *Diadegma chrysoctictos* (Gmelin) (Hymenoptera: Ichneumonidae). *Journal of Heredity* **91**, 104–111.

Butcher, R.D.J., Whitfield, W.G.F. & Hubbard, S.F. (2000b) Complementary sex determination in the genus *Diadegma* (Hymenoptera: Ichneumonidae). *Journal of Evolutionary Biology* **13**, 593–606.

Dasrat, B., Rajkumar, A., Richards-Haynes, C., Quashie-Williams, C. & Eastwood, D. (1997) Evaluation of *Allorhogas pyralophagus* Marsh (Hymenoptera: Braconidae) for the biological control of *Diatraea* spp. (Lepidoptera: Pyralidae) in sugar-cane in Guyana. *Crop Protection* **16**, 723–726.

Ellers, J., van Alphen, J.J.M. & Sevenster, J.G. (1998) A field study of size-fitness relationships in the parasitoid *Asobara tabida*. *Journal of Animal Ecology* **67**, 318–324.

Godfray, H.C.J. (1994) *Parasitoids: behavioral and evolutionary ecology*. 473 pp. Princeton, Princeton University Press.

Godfray, H.C.J. & Hardy, I.C.W. (1993) Sex ratio and virginity in haplodiploid insects. pp. 402–417 *in* Wrensch, D.L. & Ebbert, M.A. (*Eds*) *Evolution and diversity of sex ratio in insects and mites*. New York, Chapman & Hall.

Green, R.F., Gordh, G. & Hawkins, B.A. (1982) Precise sex ratios in highly inbred parasitic wasps. *American Naturalist* **120**, 653–665.

Hamilton, W.D. (1967) Extraordinary sex ratios. *Science* **156**, 477–488.

Hardy, I.C.W., Dijkstra, L.J., Gillis, J.E.M. & Luft, P.A. (1998) Patterns of sex ratio, virginity and developmental mortality in gregarious parasitoids. *Biological Journal of the Linnaean Society* **64**, 239–270.

Hartl, D.L. (1971) Some aspects of natural selection in arrhenotokous populations. *American Zoologist* **11**, 309–325.

Heimpel, G.E. (1994) Virginity and the cost of insurance in highly inbred Hymenoptera. *Ecological Entomology* **19**, 299–302.

Heinz, K.M. (1998) Host size-dependent sex allocation behaviour in a parasitoid: implications for *Catolaccus grandis* (Hymenoptera: Pteromalidae) mass rearing programmes. *Bulletin of Entomological Research* **88**, 37–45.

Hopper, K.R. & Roush, R.T. (1993) Mate finding, dispersal, number released, and the success of biological control introductions. *Ecological Entomology* **18**, 321–331.

Jones, W.T. (1982) Sex ratio and host size in a parasitic wasp. *Behavioral Ecology and Sociobiology* **10**, 207–210.

Kazmer, D.J. & Luck, R.F. (1995) Field tests of the size-fitness hypothesis in the egg parasitoid *Trichogramma pretiosum*. *Ecology* **76**, 412–425.

Martinez, A.J., Bard, J. & Holler, T.A. (1988) Mass rearing sugarcane borer and Mexican rice borer for production of parasites *Allorhogas pyralophagus* and *Rhacontus rosilensis*. USDA-APHIS-PPQ, APHIS 83–1.

Meagher, R.L., Jr., Wilson, L.T. & Pfannenstiel, R.S. (1996) Sampling *Eoreuma loftini* (Lepidoptera: Pyralidae) on Texas sugarcane. *Environmental Entomology* **25**, 7–16.

Meagher, R.L., Jr., Smith, J.W., Jr., Browning, H.W. & Saldaña, R.R. (1998) Sugarcane stemborers and their parasites in southern Texas. *Environmental Entomology* **27**, 759–766.

Melton, C.W. & Browning, H.W. (1986) Life history and reproductive biology of *Allorhogas pyralophagus* (Hymenoptera: Braconidae), a parasite imported for release against *Eoreuma loftini* (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America* **79**, 402–406.

Rodríguez del Bosque, L.A., Smith, J.W., Jr. & Browning, H.W. (1988) Damage by stalkborers (Lepidoptera: Pyralidae) to corn in northeastern Mexico. *Journal of Economic Entomology* **81**, 1775–1780.

Smith, J.W., Jr., Browning, H.W. & Bennet, F.D. (1987) *Allorhogas pyralophagus* [*Hym., Braconidae*], a gregarious external parasite imported into Texas, USA, for biological control of the stalkborer *Eoreuma loftini* [*Lep., Pyralidae*] on sugarcane. *Entomophaga* **32**, 477–482.

Smith, J.W., Jr., Wiedenmann, R.N. & Overholt, W.A. (1993) *Parasites of lepidopteran stemborers of tropical gramineous plants*. 89 pp. Nairobi, ICIPE Science Press.

van den Assem, J., van Iersel, J.J.A. & Los-den Hartogh, R.L. (1989) Is being large more important for female than for male parasitic wasps? *Behaviour* **108**, 160–195.

Visser, M.E. (1994) The importance of being large: the relationship between size and fitness in females of the parasitoid *Aphaereta minuta* (Hymenoptera: Braconidae). *Journal of Animal Ecology* **63**, 963–978.

Werren, J.H. (1993) The evolution of inbreeding in haplodiploid organisms. pp. 42–94 *in* Thornhill, N.W. (*Ed.*) *The natural history of inbreeding and outbreeding*. Chicago, University of Chicago Press.

West, S.A., Flanagan, K.E. & Godfray, H.C.J. (1996) The relationship between parasitoid size and fitness in the field: a study of *Achrysocharoides zwoelferi* (Hymenoptera: Eulophidae). *Journal of Animal Ecology* **65**, 631–639.

Youm, O., Browning, H.W. & Gilstrap, F.E. (1988) Population dynamics of stalk borers attacking corn and sorghum in the Texas Rio Grande Valley. *Southwestern Entomologist* **13**, 199–204.

Zar, J.H. (1996) *Biostatistical analysis*. 662 pp. + Apps. Upper Saddle River, Prentice Hall.

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