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Biology and field performance of *Gryon clavigrallae* (Hymenoptera: Scelionidae), an egg parasitoid of *Clavigralla* spp. (Hemiptera: Coreidae) in India

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Abstract

The biology and impact of *Gryon clavigrallae* Mineo, an egg parasitoid of *Clavigralla scutellaris* Spinola and *C. gibbosa* (Westwood), was investigated. The calculated developmental threshold temperatures for females and males were 15.6°C and 15.8°C, respectively. Emergence exceeded 94% at temperatures between 22 and 30°C. Adult females lived on average 28–96 days when fed with honey. Without food, adults lived < 6 days. Mean fecundity was 56.4 eggs per female. A significant trend of lower fecundity after longer periods of host deprivation was observed. *Gryon clavigrallae* females successfully oviposited in host eggs of all ages though eggs < 4 days old were preferred. Total host handling times were significantly longer on *C. gibbosa* eggs (23.5 min) than on *C. scutellaris* eggs (12.0 min). Females readily distinguished parasitized from non-parasitized host eggs. Superparasitism was observed when few or no unparasitized eggs were available. Eggs of the two *Clavigralla* species can be separated by surface structure and condition after eclosion. *Clavigralla scutellaris* laid significantly larger egg clusters than *C. gibbosa* (19.9 versus 10.5 eggs per cluster). *Gryon clavigrallae* was present as soon as the first *Clavigralla* spp. egg clusters were found on pigeonpea. The percentage of egg clusters parasitized increased early in the season with egg cluster density and remained high (up to 83%) despite fluctuations in host density. Overall, *G. clavigrallae* parasitized 40 and 58% of *C. gibbosa* and *C. scutellaris* eggs. The percentage of egg clusters parasitized and the number of eggs parasitized per cluster increased significantly with egg cluster size. The overall parasitoid sex ratio was highly female biased but varied with the number of eggs parasitized per cluster.

Introduction

Species of *Clavigralla* (Hemiptera: Coreidae) are among the most important insect pests of pigeonpea, *Cajanus cajan* (Fabaceae), in India and Africa. Nymphs and adults pierce the pod wall and feed on the developing seeds, reducing yields by as much as 50% (Shanower *et al.*, 1999). Three species of *Clavigralla* are associated with pigeonpea in India: *C. gibbosa* Spinola is distributed in all but the northeastern part of the country, *C. scutellaris* (Westwood) occurs in the south, and *C. orientalis* Dolling is present in the north (Dolling, 1978). Most of the published studies on *Clavigralla* spp. in India refer only to *C. gibbosa* though it is likely that a mixture of species were present in these earlier studies (Shanower *et al.*, 1999). Separating species is difficult as *Clavigralla* spp. systematics is based on adult characters (Dolling, 1978) and no keys to the egg and nymphal stages are available.

The most important group of natural enemies of *Clavigralla* spp. are egg parasitoids (Shanower *et al.*, 1999).

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Until recently, only *Gryon* (*Hadronotus*) *fulviventre* (J.C. Crawford) (as *antestiae* Dodd) (Hymenoptera: Scelionidae) was reported from *Clavigralla* spp. in India (Bindra, 1965; Rawat & Kapoor, 1968; Singh & Singh, 1987). Two other studies refer to *Gryon* sp. but provide little biological or ecological information (Nawale & Jadhav, 1978; Ombir *et al*., 1996). During a study of egg parasitism of *Clavigralla* spp. at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) near Hyderabad, Andhra Pradesh, an undescribed parasitoid was reared from eggs of *C. gibbosa* and *C. scutellaris* (Shanower *et al*., 1996). The species was described as *Gryon clavigrallae* Mineo (Mineo & Caleca, 1994).

The field performance of *G. clavigrallae* was studied by Shanower *et al*. (1996). The authors reported that from over 54,500 *Clavigralla* spp. eggs collected from pigeonpea, more than 39% were parasitized. At the end of the cropping season, up to 100% of egg clusters and up to 70% of eggs were parasitized. *Clavigralla* spp. egg clusters varied in size from 2 to 62 eggs per cluster. The percentage of both egg clusters and eggs per cluster parasitized by *G. clavigrallae* were positively correlated with egg cluster size. But no information about the host density (egg clusters per plant) was collected during this study and the *Clavigralla* species were not differentiated.

The present study was conducted to provide more information on the biology and field ecology of *G. clavigrallae*. Developmental rates, longevity, and fecundity of *G. clavigrallae* were studied and preliminary observations on host handling and host discrimination behaviour were also made. In addition, the field performance of *G. clavigrallae* in relation to host density was investigated. Data on the sex ratio of the parasitoid progeny emerging from field collected egg clusters are also presented.

Materials and methods

Insect material

Separate colonies of *C. gibbosa* and *C. scutellaris* were established from adult bugs collected in pigeonpea fields at ICRISAT field station, Hyderabad, and maintained under identical conditions. The two species were separated based on the characteristics of the pronotum (Dolling, 1978). Adults and nymphs were reared on potted pigeonpea (cv ICPL 87) plants. Plants were placed in cages in a greenhouse under ambient temperature (range: 22–28°C) and photoperiod (approx. 11:13 h light:dark). Pigeonpea pods containing egg clusters were removed daily from the adult holding cages and used for experiments, maintaining the parasitoid colony or to produce additional *Clavigralla* spp. individuals.

A colony of *G. clavigrallae* was established from field-collected parasitized *Clavigralla* spp. eggs. The identification of *G. clavigrallae* was checked against type specimens lodged in the ICRISAT collection. The parasitoids were reared in a sleeve cage (40 × 35 × 32 cm) in the laboratory (22 ± 2°C; 50 ± 10% rh) and provided with food (undiluted honey). Parasitized egg clusters were held separately and newly emerged males and females kept together to ensure that females were mated prior to use in experiments. Fresh *Clavigralla* spp. egg clusters (< 24 h old) were regularly placed in the sleeve cage to maintain the parasitoid population.

Gryon clavigrallae had earlier been described from material collected at ICRISAT (Mineo & Caleca, 1994). Voucher specimens were deposited at the British Museum (Natural History).

Differentiation between Clavigralla *spp.*

Laboratory laid eggs from both *Clavigralla* species were observed under a light microscope and scanned with a JEOL JSM 35 CF (Tokyo, Japan) scanning electron microscope. The eggs were fixed and prepared for electron microscopy by the methodology described by Reddy *et al*. (1995).

The mean number of eggs recorded from field collected egg clusters were compared between the two *Clavigralla* species using Student's *t*-test for unequal variances (Snedecor & Cochran, 1980). Sample sizes were 1110 and 605 clusters for *C. gibbosa* and *C. scutellaris*, respectively.

Development rates, longevity and fecundity

The developmental period of the immature stages of *G. clavigrallae* was studied at six constant temperatures: 15°, 20°, 22°, 25°, 27° and 30° (± 1°C) under a fixed photoperiod (12:12 h light:dark) and relative humidity (50 ± 10%). Freshly collected (< 12 h old) *Clavigralla* egg clusters were exposed to a single, mated *G. clavigrallae* female for approximately 5 h. The parasitized eggs were then transferred individually to vials and placed in incubators. A minimum of 50 parasitized eggs were held at each temperature. The eggs were observed at approximately 0800 and 1700 daily, and the date and time of emergence, and sex of the parasitoid were recorded. Linear regression (SAS Institute, 1988) of the rate of development (1/d) against temperature was used to calculate threshold temperature for immature development (Campbell *et al*., 1974).

Adult longevity was studied by placing newly emerged (< 1 day old) adult parasitoids in incubators at four temperatures: 15°, 20°, 25° and 30° (± 1°C). Individual parasitoids were kept in glass vials (80 × 30 mm) without hosts, and a minimum of 80 females and 20 males were used at each temperature. Half of the insects (40 females, 10 males) at each temperature were provided with a streak of undiluted honey for food and the other half were kept without food. Mortality was recorded daily and the honey replenished as needed. Individuals that drowned in the honey (< 5 per temperature) were not included in calculations of longevity.

The fecundity of newly emerged (< 1 day old) and mated females was measured by placing a single female in a Petri dish (9 cm diameter) at 25°C and providing at least 20 host eggs every four hours for a 12 h period. Following this a fresh cluster (< 1 day old) of at least 50 eggs was provided every day until the female died. The exposed eggs were labelled and incubated. The fecundity, pre-oviposition and oviposition periods of 18 female parasitoids were recorded.

The effect of female age on fecundity was investigated using 4, 8, 12, 16, 20, 25 and 30-day-old mated females. The females were maintained with food (undiluted honey) at 25°C prior to and during the experiment. Females were isolated in cups (11 × 6 cm) and provided with a fresh egg cluster (10–20 eggs) each day until the female died. The experiment began with eight females in each age class. Only four 20-day-old and five 12-day-old females laid eggs, while in the other age classes at least six females laid eggs. The total number of eggs parasitized by females in each age class was compared using one way analysis of variance (ANOVA) and the relationship between female age and fecundity was analysed using regression analysis (SAS Institute, 1988).

The effect of host age on parasitism was studied by collecting newly laid *C. scutellaris* egg clusters from the rearing colony every morning for seven days. This provided seven age classes of eggs ranging from < 1 to 6 days old. The eggs were held at 25°C until used in the experiment. Thirty eggs from each age class were grouped together and the groups were randomly arranged in a circle on filter paper (i.e. choice situation) in the bottom of a Petri dish (12 × 2 cm). Forty 2–3-day-old, mated female parasitoids were released into the arena, resulting in a host to parasitoid ratio of 5:1. The females were removed after 6 h and the egg clusters were placed in vials and kept at 30°C until the host or parasitoid emerged. This experiment was replicated ten times using different females in each replicate. The percentage of eggs parasitized in each treatment (host age) were transformed to arcsin √ values and analysed using one way analysis of variance (ANOVA), and means were separated using the LSD procedure (SAS Institute, 1988).

In all experiments, *Clavigralla* spp. eggs which did not hatch or produce a parasitoid were dissected to see whether they contained a parasitoid or a nymph.

Host handling and host discrimination

Host handling behaviour was observed through a binocular microscope using a cold glass fibre light source under ambient conditions (24 ± 2°C, 50 ± 10% rh). The behaviour was divided into four clearly distinguishable events: drumming, tapping, oviposition (including drilling), and marking, and the duration of each was measured with a stop watch. The behaviour of 19 females on eggs of *C. scutellaris* and ten females on eggs of *C. gibbosa* were observed. The mean time for each event and for the total handling time was calculated and compared between *C. scutellaris* and *C. gibbosa* females using the Student's *t*-test for unequal variances.

The ability to discriminate between parasitized and unparasitized eggs was investigated using 2–3-day-old, mated female parasitoids. These females were placed on host eggs until oviposition was observed (approx. 10–15 min) to gain experience prior to the experiment. Egg clusters of 10–15 eggs, < 3 days old, were used in the experiment. A female was placed on the egg cluster and allowed to parasitize 50% of the eggs. Two situations were investigated: (i) the original (first) female was allowed to remain on the egg cluster, or (ii) the female was replaced with a second female. Superparasitism was compared using the ratio of 'acceptances' (insertion of the ovipositor) to 'contacts' (antennation of the egg surface) by the same or by conspecific females. The acceptance/contact (A/C) ratio was calculated from ten females for each situation. An A/C ratio close to 1 indicates that a female oviposited into nearly all of the eggs contacted. The A/C ratios for parasitized and unparasitized eggs were compared between first and second females. A 2 × 2 contingency table for the distribution of acceptance and contacts was constructed and differences in A/C ratios were evaluated using a χ^2-test. Eggs which were superparasitized were dissected to determine the number of *G. clavigrallae* eggs present.

Field trials

Two field trials using pigeonpea cultivar ICPL 87 were conducted on black vertisols at the ICRISAT research station during the 1995 (season 1) and 1996/97 (season 2) cropping seasons. Field size was approximately 1 ha in both seasons. To evaluate *Clavigralla* spp. egg cluster density, 160 pigeonpea plants were randomly sampled each week. If less than 20 egg clusters were collected, additional randomly selected plants were sampled to obtain a minimum of 20 egg clusters. To calculate the percentage of egg clusters parasitized, eggs of *C. gibbosa* and *C. scutellaris* were left unseparated as host density was relatively low and both species were equally accepted by *G. clavigrallae* (Madhuri, 1997). All egg clusters were kept separately under ambient conditions in the laboratory. The *Clavigralla* species was identified for all egg clusters collected during season 2 and for some clusters collected during season 1. After all the emerged nymphs or parasitoids had died, the following parameters were assessed for each egg cluster: (i) number of eggs per cluster, (ii) number of eggs parasitized, (iii) sex of the emerged parasitoids, and (iv) number of eggs which failed to hatch. Unhatched eggs were dissected. Eggs in which neither a dead parasitoid nor a dead nymph was found were considered infertile. The two sexes of *G. clavigrallae* can be easily separated by abdominal coloration. The abdomen of females is bright yellow, while that of males is black (Mineo & Caleca, 1994).

There are several problems associated with the term 'percentage parasitism' (van Driesche, 1983). In this study, the percentage of egg clusters parasitized has been estimated by simply dividing the number of clusters parasitized by the total number of clusters collected. All parasitism levels reported here are therefore likely to be an overestimate as parasitized *Clavigralla* spp. eggs stay longer in the field (this study) than unparasitized eggs (Bindra, 1965; Ombir *et al.*, 1992). We tried to minimize this effect by collecting only egg clusters from which no nymph or parasitoid had emerged.

To evaluate the effect of egg cluster size on: (i) cluster parasitism, (ii) the number of eggs parasitized per cluster, and (iii) the percentage of eggs parasitized per cluster, and the relationship between the number of eggs parasitized per cluster and parasitoid sex ratio, data were pooled for both seasons as there is no reason to expect differences in these parameters between seasons.

The relationship between egg cluster size and cluster parasitism (%) was analysed separately for each *Clavigralla* species based on a total of 1100 and 602 clusters for *C. gibbosa* and *C. scutellaris*, respectively. A logistic regression was used to analyse the effects of egg cluster size, *Clavigralla* species, and their interactions on cluster parasitism (SYSTAT, 1997). The relationship between egg cluster size and the number of eggs parasitized per cluster was analysed for 'discovered' clusters, i.e. clusters with at least one parasitized egg, using weighted regression analysis separately for both *Clavigralla* species after both the dependent and independent variables were √ transformed. The relationship between egg cluster size and the percentage of eggs parasitized per cluster was analysed for discovered clusters using weighted regression analysis separately for both *Clavigralla* species after the percentage data were arcsin √ transformed. If a correlation was found, the slope of the regression equations were compared between the two *Clavigralla* species using the method of Zar (1999).

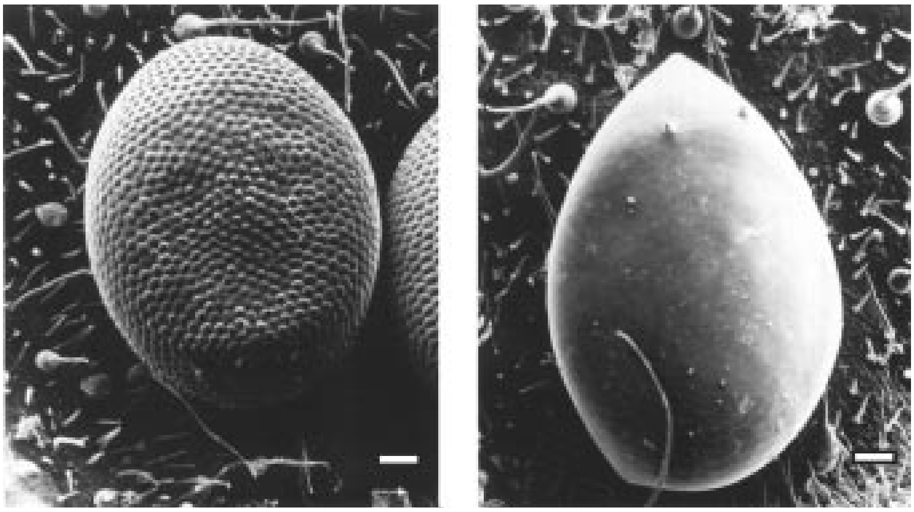

Fig. 1. Eggs of *Clavigralla gibbosa* (left) and *C. scutellaris* (right). Scale bar on both plates = 100 µm.

Progeny sex ratio data were transformed to arcsin $\sqrt{}$ values. The mean sex ratio of the parasitoids which emerged from *C. gibbosa* and *C. scutellaris* eggs were compared using Student's *t*-test. The relationship between the number of eggs parasitized per cluster and progeny sex ratio was analysed using weighted regression analysis for each *Clavigralla* species separately (SYSTAT, 1997).

Results

Differentiation between Clavigralla *spp.*

Eggs of the two *Clavigralla* spp. have very different surface structures and are easily separated. *Clavigralla gibbosa* eggs are roughly sculptured while *C. scutellaris* eggs are smooth and shiny (fig. 1). This difference is readily visible with a hand lens. After a *C. gibbosa* nymph emerges, the operculum is lifted but the egg retains its oval shape. In the case of *C. scutellaris* eggs, the lateral walls of the egg and the operculum roll together, and the operculum often separates from the rest of the egg shell.

Field collected egg clusters of *C. gibbosa* contained 1–31 eggs whereas clusters of *C. scutellaris* contained 1–50 eggs (fig. 2). The average (± SE) egg cluster size of *C. gibbosa* was 10.5 ± 0.13 eggs. The average egg cluster size of *C. scutellaris* was significantly larger with 19.9 ± 0.33 eggs ($t = -26.2$, df = 808, $P < 0.001$; Student's *t*-test for unequal variances).

Development rates, longevity and fecundity

No development was observed at 15°C. More than 94% of parasitoids emerged at all other temperatures except 20°C, at which only 46.2% of the parasitoids completed development (table 1). Egg to adult development time ranged from approximately 10 days at 30°C to more than 36 days at 20°C. Calculated threshold temperatures were 15.8°C for males ($y = -0.114 + 0.0072x$, $R^2 = 0.99$, $P < 0.05$) and 15.6°C for females ($y = -0.104 + 0.0066x$, $R^2 = 0.99$, $P < 0.05$). The average thermal requirements in degree-days were calculated for males and females using the threshold temperatures. Female degree-day requirements were approximately 10% higher than males (table 1).

Adult *G. clavigrallae* are long lived when supplied with honey in the absence of hosts. Females with food lived at least 28 days and lived longer than males at all temperatures (table 2). Males and females lived only 1–6 days without food. At 25°C a pre-ovipositional period of 6–48 h (mean ± SE; 17.0 ± 2.3) was observed and females laid eggs over a

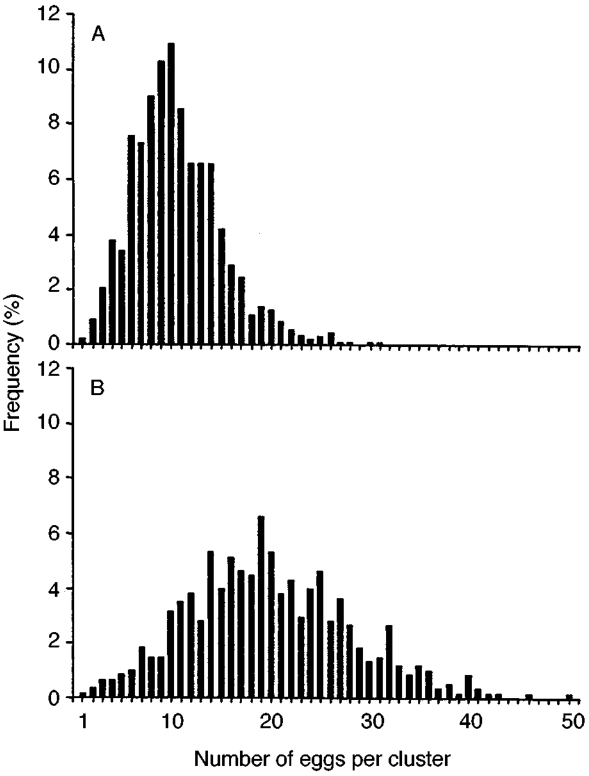

Fig. 2. Frequency distribution of (A) *Clavigralla gibbosa* (n = 1110) and (B) *C. scutellaris* (n = 605) egg cluster sizes.

Table 1. Mean (± SE) egg to adult developmental time (days), degree-days above threshold temperature, and percentage emergence of male and female *Gryon clavigrallae* at five constant temperatures.

Temp. (°C)	Sex	n	Development time	Degree-days	Emergence (%)[1]
20	♂	9	36.4 ± 0.18	152.9	
	♀	15	38.5 ± 0.20	169.6	46.2
22	♂	15	20.1 ± 0.06	124.6	
	♀	40	21.4 ± 0.04	136.9	96.5
25	♂	20	14.7 ± 0.02	135.2	
	♀	50	15.8 ± 0.03	148.7	94.6
27	♂	20	12.7 ± 0.05	142.2	
	♀	55	13.5 ± 0.04	153.9	97.4
30	♂	20	9.6 ± 0.02	136.3	
	♀	50	10.5 ± 0.03	152.6	97.2

[1] Per cent successful emergence of both males and females combined.

Table 2. Mean (± SE) longevity (days) of male and female *Gryon clavigrallae* with and without food at four constant temperatures.

Temp. (°C)	Sex	Fed[1]	Range	Unfed	Range
15	♂	89.6 ± 5.69	68–120	5.7 ± 0.84	3–9
	♀	95.7 ± 2.70	54–123	5.5 ± 0.43	3–8
20	♂	80.9 ± 4.49	62–90	3.9 ± 0.54	2–7
	♀	89.4 ± 4.30	67–127	4.0 ± 0.31	2–7
25	♂	28.8 ± 5.34	6–57	1.9 ± 0.22	1–3
	♀	55.0 ± 3.30	6–88	2.1 ± 0.15	1–4
30	♂	14.1 ± 2.44	5–37	1.1 ± 0.07	1–2
	♀	28.3 ± 2.70	5–57	1.3 ± 0.08	1–2

[1] n = 80 females (40 fed, 40 unfed) and 20 males (10 fed, 10 unfed) at each temperature.

period of 6–24 (mean: 15.1 ± 1.3) days. Fecundity of newly emerged and mated females ranged from 26 to 90 eggs (mean: 56.4 ± 4.4) with most eggs laid 2–6 days after emergence (fig. 3). More than half (58%) of the eggs were laid within the first 6 days and 83% of the eggs were laid within 12 days after emergence.

Fecundity was significantly different (ANOVA; $F_{6,40} = 2.99$, $P < 0.05$) among females deprived of hosts for up to 30 days (fig. 4). Fecundity ranged from 16.8 eggs per female for 30-day-old females to 46.5 eggs per female for 4-day-old females. A significant trend of lower fecundity for older females (i.e. females deprived of hosts for longer periods) was observed ($y = -1.068 + 51.8x$, $R^2 = 0.25$, $P < 0.01$). Considering the low number of females used in this experiment these results must be considered preliminary.

Gryon clavigrallae attacked host eggs of all ages, though eggs of 4 days and older were infrequently parasitized (fig. 5). Differences in parasitization rates for eggs of different ages were highly significant (ANOVA; $F_{6,69} = 14.0$, $P < 0.00001$).

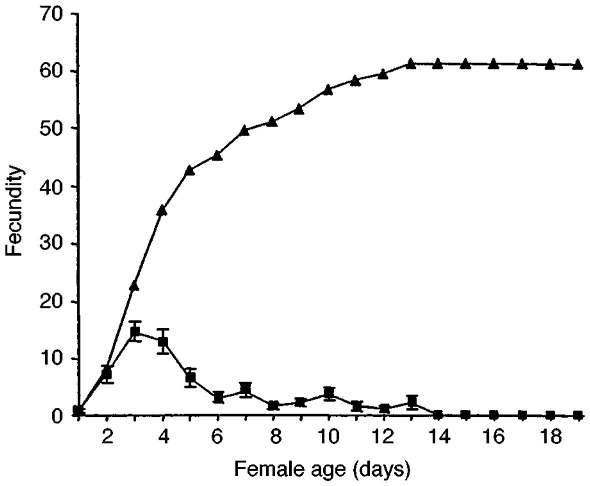

Fig. 3. Mean daily (■; ± SE) and cumulative (▲) fecundity of *Gryon clavigrallae* at 25°C (n = 18).

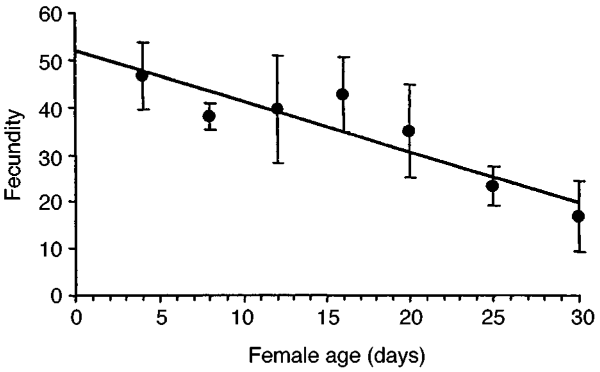

Fig. 4. Mean (± SE) fecundity of *Gryon clavigrallae* females deprived of hosts for different lengths of time ($y = -1.068 + 51.8x$, $R^2 = 0.25$).

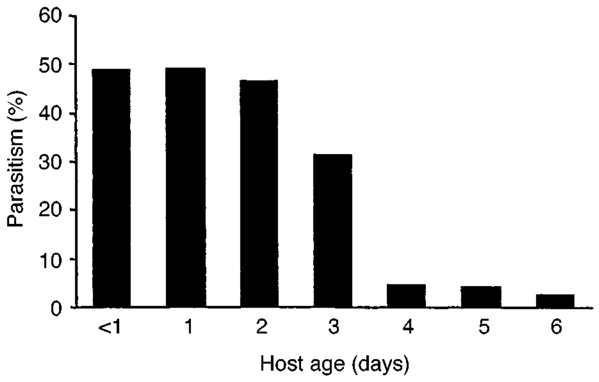

Fig. 5. Percentage of *Clavigralla scutellaris* eggs of differing age parasitized by female *Gryon clavigrallae* (n = 10).

Host handling and host discrimination

Gryon clavigrallae females took approximately twice as long to parasitize *C. gibbosa* eggs as *C. scutellaris* eggs (table 3). Among the four host handling events observed, oviposition (including drilling) required the most time with more than 87% of the total handling time on *C. scutellaris* eggs and more than 91% of the total handling time on *C. gibbosa* eggs. Drumming and oviposition events were significantly longer on *C. gibbosa* eggs than on *C. scutellaris* eggs.

Superparasitism was rare and generally occurred when unparasitized eggs were unavailable (table 4). The first female on the egg cluster quickly oviposited in unparasitized eggs (A/C ratio = 0.97) and only after many contacts, accepted previously parasitized eggs (A/C ratio = 0.02). The second female on an egg cluster accepted unparasitized eggs at approximately the same rate (A/C ratio = 0.92) as the first female. The second female superparasitized eggs significantly more frequently (A/C ratio = 0.07) than the first female. The majority (nine of 15) of the eggs superparasitized by the second female were conspecific superparasitism (table 4). Ten of the 19 superparasitized eggs were dissected and all of these contained two *G. clavigrallae* eggs. The other half were incubated and produced only a single *G. clavigrallae* parasitoid. All of the 19 cases of superparasitism occurred when less than 15% of the eggs in a cluster were unparasitized.

Table 3. Mean (± SE) handling time (min.) for female *Gryon clavigrallae* on eggs of *Clavigralla scutellaris* (n = 19) and *C. gibbosa* (n = 10).

Event	*C. scutellaris*	*C. gibbosa*	P^1
Drumming	0.43 ± 0.03	0.65 ± 0.04	**
Tapping	0.22 ± 0.01	0.27 ± 0.03	NS
Oviposition	10.5 ± 0.80	21.6 ± 2.4	**
Marking	0.86 ± 0.06	0.98 ± 0.11	NS
Total handling time	12.0 ± 0.84	23.5 ± 2.5	**

[1] Means within the same row followed by ** are significantly different ($P < 0.001$; Student's *t*-test for unequal variances); NS = not significant ($P > 0.05$).

Field trials

Clavigralla spp. started ovipositing on pigeonpea as soon as the first pods appeared. *Clavigralla scutellaris* entered the field earlier than *C. gibbosa*, but the latter species was predominant by the end of the cropping season. More than 98% of all *Clavigralla* spp. egg clusters were collected from pigeonpea pods; few clusters were found on leaves.

During both seasons, *G. clavigrallae* was present in the pigeonpea field as soon as the first *Clavigralla* spp. egg clusters were found (fig. 6). *Gryon clavigrallae* was the only parasitoid reared from the egg samples. Early in the season, cluster parasitism increased continuously over a period of five weeks. Three to four weeks after *Clavigralla* spp. started ovipositing on pigeonpea, more than 50% of the egg clusters were parasitized (fig. 6). Despite considerable fluctuations in host density, cluster parasitism then remained above 51% reaching a maximum of 83%.

The logistic regression analysis revealed that egg cluster size significantly affected cluster parasitism which increased with increasing cluster size ($G = 10.80$, $P < 0.001$). The two *Clavigralla* species showed a different cluster parasitism ($G = 6.72$, $P < 0.01$), but there was no significant cluster size–*Clavigralla* species interaction ($G = 1.18$, $P > 0.25$), indicating that the parasitoids' response to increasing cluster size was similar for the two host species.

For egg clusters discovered by *G. clavigrallae*, the number of eggs parasitized per cluster increased linearly with egg cluster size for both *C. gibbosa* ($y = 0.776 + 0.515x$, $R^2 = 0.158$, $P < 0.0001$; n = 737) and *C. scutellaris* ($y = 0.185 + 0.835x$, $R^2 = 0.413$, $P < 0.0001$; n = 459) (fig. 7). However, the slope of the regression lines differs significantly between the two species,

Table 4. Acceptance of parasitized and unparasitized host eggs by first and second female *Gryon clavigrallae*.

Condition of egg	Female[1]	Eggs accepted	Number of contacts	A/C[4] ratio	χ^2	*P*
Unparasitized	First	62	64	0.97	1.69	$0.25 < P < 0.1$
Unparasitized	Second	78	85	0.92		
Parasitized	First	4[2]	174	0.02	5.52	$0.025 < P < 0.01$
Parasitized	Second	15 (9c, 6s)[3]	194	0.07		

[1] First = first female allowed to continue ovipositing after half of the eggs in a cluster were parasitized; Second = first female replaced after half of the eggs in a cluster were parasitized.

[2] All self-superparasitized by first female.

[3] Both conspecific superparasitized (9c) and self-superparasitized (6s) by second female.

[4] A/C denotes the ratio of acceptances (insertion of ovipositor) to contacts (antennation of the egg surface).

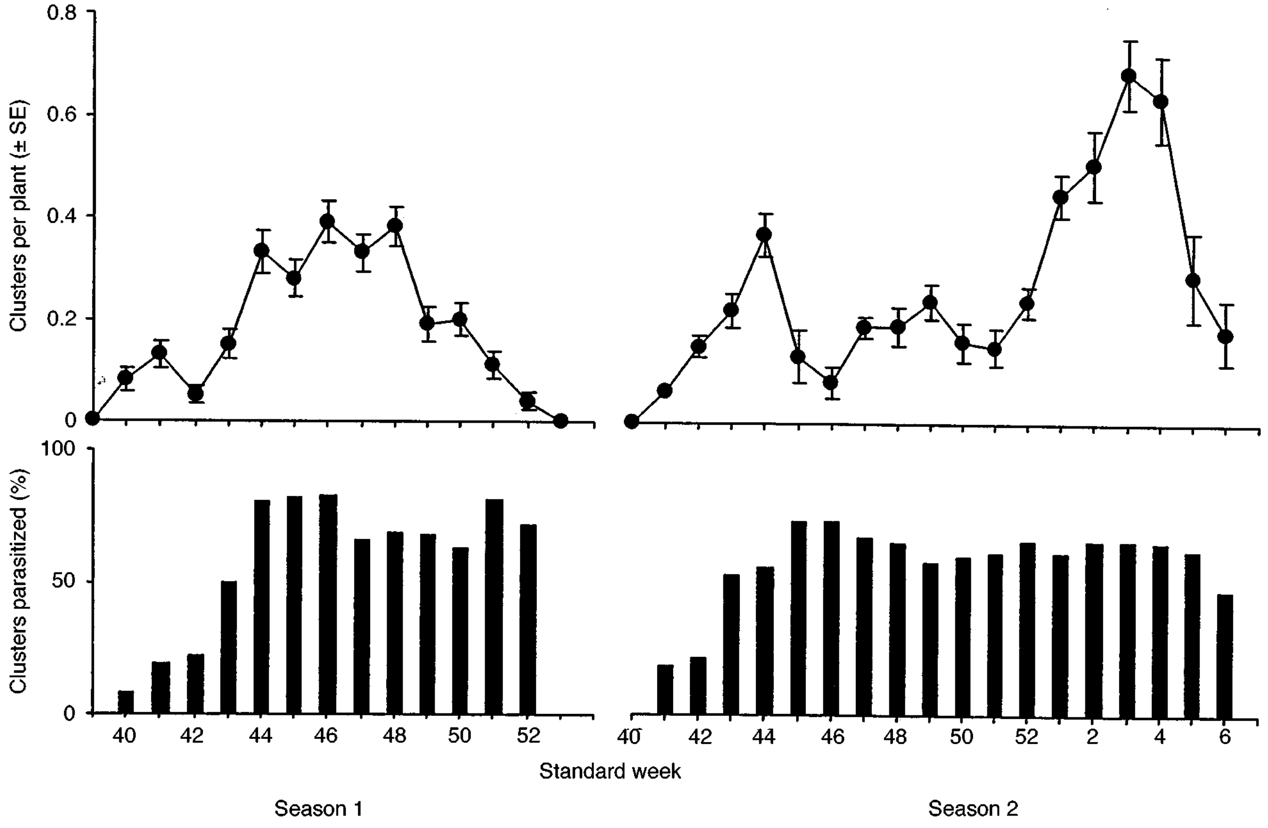

Fig. 6. *Clavigralla* spp. egg cluster density (mean ± SE) on pigeonpea and percentage of egg clusters parasitized by *Gryon clavigrallae* during the 1995 (season 1) and 1996/97 (season 2) cropping seasons. Standard week refers to weeks 1–52 of the annual year.

with *C. scutellaris* showing a stronger increase ($F = 141.95$, $P < 0.001$). The percentage of eggs parasitized per cluster decreased significantly with increasing cluster size for *C. gibbosa* ($y = 64.743 - 0.90x$, $R^2 = 0.029$, $P < 0.001$), but no relationship was observed for *C. scutellaris* ($P = 0.183$).

From a total of more than 11,500 eggs collected from each species, 43% of *C. gibbosa* and 62% of *C. scutellaris* eggs did not produce a nymph (table 5). The most important mortality factor was parasitism by *G. clavigrallae*, causing 40 and 58% mortality of eggs of *C. gibbosa* and *C. scutellaris*, respectively. The mortality of the developing parasitoids and nymphs and the percentage of infertile eggs was higher for *C. scutellaris* than for *C. gibbosa*.

The progeny sex ratio of *G. clavigrallae* was on average highly female biased. There was a significant difference in the sex ratio (mean ± SE) of the progeny emerging from *C. gibbosa* and *C. scutellaris* eggs which were 0.33 ± 0.010 (n = 675) and 0.25 ± 0.010 (n = 414), respectively ($t = 3.36$, df =1087, $P < 0.001$). The sex ratio varied with the number of eggs parasitized per egg cluster, and was similar for eggs of the two *Clavigralla* species (fig. 8). The maximum proportion of males was recorded from egg clusters which produced three parasitoids (0.44 and 0.42 for *C. gibbosa* and *C. scutellaris*, respectively). The weighted regression analysis revealed a significant decrease in sex ratio with an increase in the number of eggs parasitized per cluster for *C. gibbosa* ($y = 0.402 - 0.01x$, $R^2 = 0.044$, $P < 0.001$) but the relationship for *C. scutellaris* was not significant ($P = 0.80$). The smaller sample size, and consequently larger error bars, for *C. scutellaris* eggs (fig. 8) may be partly responsible for the lack of significant relationship in this species.

Discussion

Gryon clavigrallae was reared from field-collected eggs of *Clavigralla gibbosa* and *C. scutellaris*, and readily attacked eggs of both species in the laboratory. In addition, eggs of *Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae) collected from the field were also parasitized by *G. clavigrallae* (Madhuri, 1997). Earlier reports of *Gryon* sp. parasitizing eggs of *C. gibbosa*, *N. viridula* and another pentatomid, *Acrosternum graminea* (Fabricius) in India (Nawale & Jadhav, 1978; Yadava *et al.*, 1982; Velayudhan & Senrayan, 1989; Ombir *et al.*, 1996) may refer to *G. clavigrallae*.

Gryon clavigrallae appears well synchronized with its hosts. Egg to adult development time for female *G. clavigrallae* is 10.5 days at 30°C and 15.8 days at 25°C, while *C. gibbosa* completes development in 16.6 days at 30°C and 22.7 days at 25°C (Ombir *et al.*, 1992). The faster development time allows the parasitoid to respond quickly to changes in host density. In addition, *G. clavigrallae* adults are long-lived, surviving for three months or more when fed honey in the absence of hosts. Longevity in the field is likely to be shorter, though scelionid adults are known to be relatively long-lived when compared to other parasitoids

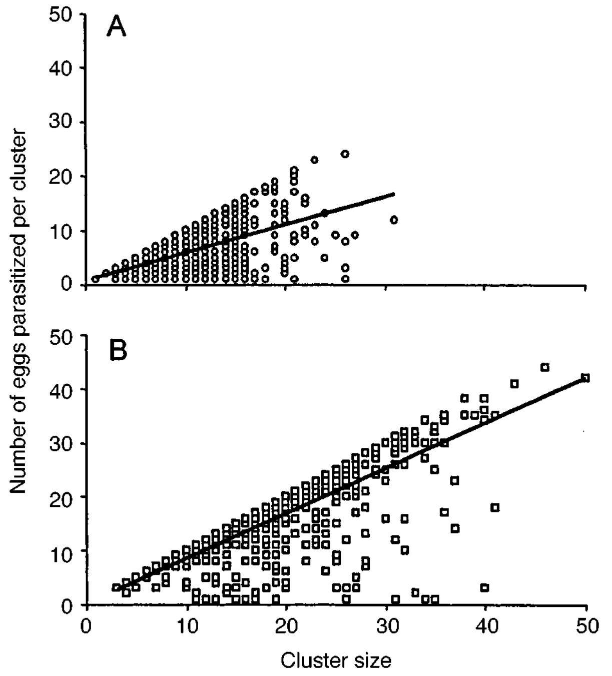

Fig. 7. Relationship between (A) *Clavigralla gibbosa* ($y = 0.776 + 0.515x$, $R^2 = 0.16$, $n = 737$) and (B) *C. scutellaris* ($y = 0.185 + 0.835x$, $R^2 = 0.41$, $n = 459$) egg cluster size and the number of eggs parasitized per cluster for discovered clusters.

(Orr, 1988). The long adult lifespan allows *G. clavigrallae* populations to survive periods of reduced host availability. In India this occurs during the cooler and drier times of the year when *Clavigralla* spp. populations are lower (Mishra & Odak, 1981). *Gryon clavigrallae* did not develop well at temperatures below 20°C. This however, is unlikely to constrain parasitoid populations in India because daily mean temperatures are rarely below 20°C during the pigeonpea season.

Gryon clavigrallae females parasitized host eggs of all ages though a clear preference for eggs < 4 days old was observed. A preference for younger host eggs has been noted for other *Gryon* species, including *G. fulviventre* (as *G. antestiae*), *G. nixoni* Masner and *G. japonicum* (Ashmead) (Hymenoptera: Scelionidae) (Singh & Singh, 1987; Morrill & Almazon, 1990; Noda, 1993). Host deprivation had an adverse effect on *G. clavigrallae* fecundity, with 25 and 30-day-old females laying significantly fewer eggs than the youngest females. But for shorter periods of host deprivation, *G. clavigrallae* was able to maintain its reproductive capacity. If this is due to cyclical egg resorption or to good egg retention capability, is not known. The ability to maintain reproductive capacity together with the parasitoid's long life-span should make *G. clavigrallae* an important biological control agent (Doutt, 1964). Other scelionids react variably to host deprivation: fecundity of *Telenomus busseolae* (Gahan) (Hymenoptera: Scelionidae) was not affected by host deprivation for periods of up to 10 days (Olaye *et al.*, 1997), while fecundity of *Gryon pennsylvanicum* (Ashmead) (Scelionidae) was significantly lower when hosts were withheld for 3 day intervals (Vogt & Nechols, 1993).

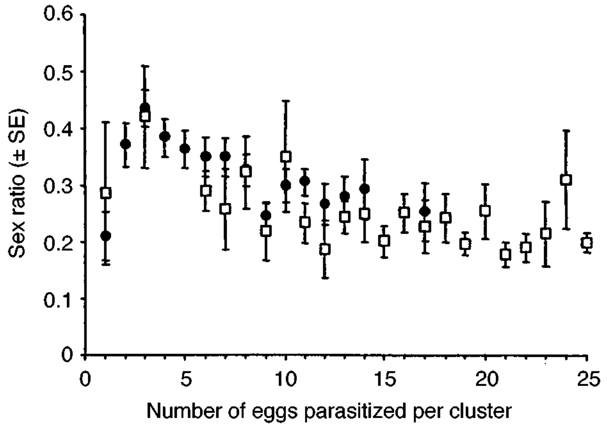

Fig. 8. Relationship between the number of eggs parasitized per cluster and *Gryon clavigrallae* progeny sex ratio (mean proportion of males ± SE) for (●) *Clavigralla gibbosa* ($10 \le n \ge 90$) and (□) *C. scutellaris* ($10 \le n \ge 23$).

Gryon clavigrallae females readily distinguish parasitized and unparasitized host eggs. Similar observations have been made for other scelionid egg parasitoids (Waage, 1982). Acceptance of parasitized host eggs was 3-fold higher by conspecific females than by the same female. Superparasitism of eggs parasitized by conspecifics is adaptive when the quantity of unparasitized eggs is low (Van Alphen & Visser, 1990; Godfray, 1994). However, it is interesting that in our study more than one third of the superparasitism events by conspecific *G. clavigrallae* were self-superparasitism and not ovipositions into eggs parasitized by the first female. A complicating factor in the design of this experiment is the possibility of higher egg load in the second female compared to the first female

Table 5. Egg parasitism of *Clavigralla* spp. on pigeonpea by *Gryon clavigrallae*.

		Parasitized eggs			Unparasitized eggs			
Clavigralla species	Number of eggs collected	Total	Live parasitoids	Dead	Total	Live nymphs	Dead	Infertile
C. gibbosa	11,699	4680	4163	517	7019	6661	328	30
			(35.6)[1]	(4.4)		(56.9)	(2.8)	(0.3)
C. scutellaris	12,722	7327	6076	1251	5395	4774	532	89
			(47.8)	(9.8)		(37.5)	(4.2)	(0.7)

[1] Percentage values are given in parentheses

(Minkenberg *et al*., 1992). The preliminary observations made in this study need to be supplemented with more detailed observations, including field studies, to better understand the host discrimination ability of *G. clavigrallae*.

During the field experiments, only *C. gibbosa* and *C. scutellaris* were found on pigeonpea. The eggs of the two species are easily differentiated by their surface structure and the condition of the eggs after eclosion. With few exceptions, published studies in India refer only to *C. gibbosa* though other *Clavigralla* species may have been present simultaneously. For example, Singh & Patel (1968) describe the eggs of *C. gibbosa* as having a smooth chorion and splitting longitudinally at eclosion. We believe that these authors were referring to *C. scutellaris*, as eggs of this species have a smooth surface and the lateral walls of the egg roll together after a nymph has emerged, giving the appearance of a longitudinal opening. The eggs of *C. gibbosa* retain their form after eclosion, suggesting a thicker chorion than *C. scutellaris* eggs. Further evidence for this is that the handling time of *G. clavigrallae*, i.e. the drilling/oviposition event, is significantly longer on *C. gibbosa* eggs than on *C. scutellaris* eggs.

Clavigralla spp. were found in the field as soon as the first pods were available. The bugs showed a strong ovipositional preference for pods as has been reported earlier (Bindra, 1965; Singh & Patel, 1968). The egg parasitoid *G. clavigrallae* appeared simultaneously with the hosts. The percentage of egg clusters that were parasitized increased with host density over a period of about five weeks and then remained between 51 and 83% independent of the host density. A similar trend reaching up to 100% cluster parasitism has been reported by Shanower *et al*. (1996). *Gryon clavigrallae* is very efficient in locating hosts as evidenced by the large percentage of egg clusters parasitized at the low host densities observed during our study. For comparison, much higher infestation levels of *Clavigralla* spp. on pigeonpea have been found in earlier studies (Materu, 1971; Mishra & Odak, 1981; Dreyer, 1994). The increase in the percentage of egg clusters parasitized during the first few weeks of the season could be due to an increase in the parasitoid population as the generation time of *G. clavigrallae* at field temperatures is approximately two weeks and/or due to a functional or aggregation response. Dreyer (1994) found no relationship between *C. tomentosicollis* Stål (Hemiptera: Coreidae) density (eggs per plant) on cowpea and the percentage of eggs parasitized by *G. fulviventre* and *Ooencyrtus utetheisae* (Risbec) (Hymenoptera: Encyrtidae).

Our study, and an earlier one (Shanower *et al*., 1996), observed that the percentage of *Clavigralla* spp. egg clusters parasitized by *G. clavigrallae* increased with egg cluster size. This relationship suggests that larger egg clusters are found more easily by the parasitoids. The number of eggs parasitized per cluster also increased with egg cluster size. The number of eggs parasitized in large clusters was well beyond the maximum daily fecundity of 15 eggs per female (fig. 3), suggesting that these clusters were visited by more than a single female or that individual females stayed on or near a cluster for several days. In addition, *G. clavigrallae* may show a functional response to changing host densities as has been observed for *G. fulviventre* [as *G. gnidus* (Nixon)] (Umeh & Ofoegbu, 1983). The relationship between the number of eggs parasitized per cluster, and the percentage of eggs parasitized per cluster for the two *Clavigralla* species are linked. The slope in the number of eggs parasitized per cluster increases much more quickly for *C. scutellaris* egg clusters than for *C. gibbosa* egg clusters (fig. 7), while the percentage of eggs parasitized per cluster declines in *C. gibbosa* clusters. There is no relationship between the percentage of eggs parasitized per cluster and cluster size in *C. scutellaris* clusters. Two factors contribute to this: (i) the generally larger egg cluster size for *C. scutellaris*, and (ii) the shorter handling time required to parasitize this species as compared to *C. gibbosa*. *Gryon clavigrallae* is able to parasitize more *C. scutellaris* eggs than *C. gibbosa* eggs in a given amount of time. Similar to our results, *C. tomentosicollis* egg cluster size and the percentage of egg clusters parasitized by *G. fulviventre* and *O. utetheisae* were positively correlated and the percentage of eggs parasitized per cluster decreased with increasing egg cluster size (Dreyer, 1994). A positive relationship between egg cluster size and cluster parasitism has also been reported for other egg parasitoids including *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) (Van der Schaaf *et al*., 1984). In contrast, Taylor (1975) did not find a significant relationship between *C. tomentosicollis* egg cluster size and cluster parasitism by *G. fulviventre* [as *G. gnidus*].

Gryon clavigrallae is an important mortality factor for both *Clavigralla* species. However, the impact on the pests' population dynamics is not clear. The parasitoid attacked fewer eggs of *C. gibbosa* compared to *C. scutellaris* probably because the latter species laid larger egg clusters which were more easily found. It is likely that the longer handling times on *C. gibbosa* eggs may also contribute to the lower egg parasitism level observed in this species. The mortality of the developing parasitoids was higher in eggs of *C. scutellaris* indicating that this species might be a less suitable host for the development of *G. clavigrallae*. It is possible that the two *Clavigralla* species followed different evolutionary lines to defend against egg parasitism. *Clavigralla gibbosa* developed a harder egg chorion, while *C. scutellaris* defends against parasitism by laying larger egg clusters. Godfray (1987) has discussed how parasitoid attack can select for the production of large egg clusters.

The overall progeny sex ratio produced by *G. clavigrallae* was highly female biased but within the range reported for other 'quasi-gregarious' scelionids attacking gregarious hosts (Waage, 1982). Male *G. clavigrallae* emerge shortly before the females and sib-mating is likely. Therefore, local mate competition is expected to be of importance for *G. clavigrallae*. As discussed above, it is likely that larger egg clusters were visited by more than one parasitoid female, leading to an increased number of eggs parasitized per cluster. Following the demands of the local mate competition model proposed by Hamilton (1967), the progeny sex ratio (proportion of males) should increase with the number of females parasitizing an egg cluster (Waage, 1986; Godfray, 1994). But the opposite was found in this study as the proportion of males decreased with an increase in the number of eggs parasitized per egg cluster. Our results are similar to laboratory studies in which individual, solitary egg parasitoids were offered a cluster of hosts, and allocate a maximum of male progeny early in the oviposition bout (see Godfray, 1994, for review). There are two possible explanations for this discrepancy: (i) *G. clavigrallae* does not adjust its sex allocation in response to any cues indicating the (former) presence of conspecifics on the egg cluster, which would be in contrast to most other parasitoids which produce an increased proportion of male progeny if conspecifics are or were present (Godfray, 1994); or (ii) large egg clusters were parasitized by only one female which defended it against conspecifics, a behaviour observed in other scelionids (Waage, 1982). The observed change in sex ratio with the number of eggs parasitized could also explain why the average sex ratio was higher in progeny emerging from *C. gibbosa* eggs when compared to *C. scutellaris* eggs, as clusters of *C. gibbosa* contained on average fewer parasitized eggs. More laboratory studies are needed to understand the sex allocation behaviour of *G. clavigrallae* on the different host species.

Gryon clavigrallae females are long lived, have good host locating and discriminating ability, relatively short handling times and can maintain their reproductive potential over periods of host deprivation. Field studies have documented high rates of parasitism of *Clavigralla* spp. eggs in India. This parasitoid appears to be an efficient and effective natural enemy which could be considered for use in other locations or against other *Clavigralla* species. A lifetable study of *Clavigralla* spp. would provide useful information on the relative impact of *G. clavigrallae* and its potential as a biological control agent.

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