Indian Journal of Experimental Biology
Vol. 49, October 2011, pp. 786-790

Effect of mating and parasitism regimes on progeny production and sex-ratio of *Campoletis chlorideae* Uchida

M K Dhillon* & H C Sharma

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, India

Received 25 April 2011; revised 20 June 2011

The ichneumonid parasitoid, *C. chlorideae* is an important natural enemy of pod borer/bollworm, *Helicoverpa armigera* (Hubner) in different agro-ecosystems. The sex-ratio of parasitoids has an important bearing on the population build up of the natural enemies for biological control of insect pests. Therefore, the present studies were conducted to gain an understanding of the influence of mating behaviour and abundance of the insect host on fecundity and sex-ratio of the parasitoid, *C. chlorideae*. There was no significant influence of number of matings and abundance of the insect host on cocoon formation, adult emergence, and larval and pupal periods of *C. chlorideae*. However, fecundity and female longevity were significantly influenced by mating and abundance of the insect host. There was a significant and positive correlation (r = 0.84**) between longevity and fecundity of *C. chlorideae* females. The unmated *C. chlorideae* females produced only males. Nearly 20% of the females that had mated twice were able to parasitize the *H. armigera* larvae successfully. The sex-ratio of the progeny from females that had mated twice was male biased. Females mated with males from the unmated females produced significantly less numbers of females than those mated with males from the fertilized females, indicating genetic regulation of sex-ratio in *C. chlorideae*.

Keywords: *Campoletis chlorideae*, Courtship behaviour, *Helicoverpa armigera*, Oviposition behaviour, Parasitoid, Progeny production, Sex-ratio

The ichneumonid parasitoid, *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae) is one of the most important natural enemies of the noctuid, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), and other lepidopteran insects in different agro-ecosystems[1-3]. The parasitism of *H. armigera* larvae by *C. chlorideae* under natural conditions has been reported to be 44.2, 33.1, 32.6, 11.1, 7.1, and 4.2% in sorghum, chickpea, pearl millet, cotton, groundnut, and pigeonpea, respectively[1,4]. It prefers to parasitize the late second-instar (4 to 5-day-old) larvae of *H. armigera*[5]. After parasitization, the egg hatches in 1.0 to 1.5 days inside the host larva, and the parasitoid maggot feeds on the contents of the host larva, and completes development in 6 to 8 days[6]. On completion of development, the parasitoid larva emerges from the host larva, killing the latter in the process. It then weaves a cocoon, and the pupal period lasts for 5 to 7 days. Total development is completed in 12 to 16 days at 27° ± 2°C, and 65 - 75% RH[6,7].

Although, there is a significant correlation between the age of the host larvae and percent parasitism[8], parasitism potential of *C. chlorideae* also varies across climatic conditions, and crop and insect hosts[3,9]. Information on regulation of sex-ratio in *C. chlorideae* has an important bearing on the use of this parasitoid for biological control of lepidopteran insects. The age of males and females of *C. chlorideae* at the time of mating, and host densities have earlier been reported to influence sex-ratio[10-12]. Although several sex determination mechanisms have been suggested in the hymenopteran parasitoids, most of these are species specific, and many of them have not been supported with strong evidence[13-17]. There is no clear understanding of the mechanism of sex regulation in *C. chlorideae*. Although mating in *C. chlorideae* with single male is well established, copulation of two males simultaneously with a single female was also observed as has earlier been reported in case of *Campoletis sonorensis*[18]. However, the information on influence of such courtship behaviour on progeny production and sex-ratio is unavailable in case of *C. chlorideae*. Therefore, we studied the

---

*Correspondent author's present address
Division of Entomology
Indian Agricultural Research Institute (IARI)
New Delhi 110 012, India
Telephone: (91)-11-25842482
Fax: (91)-11-25842482
E-mail: mukeshdhillon@rediffmail.com

influence of mating and host abundance on progeny production and sex-ratio in *C. chlorideae*.

Materials and Methods

Insect culture—*H. armigera* larvae reared on chickpea based semi-synthetic artificial diet were used as a host for raising *C. chlorideae* for bioassays (27°±2°C, 65-85% RH, and 12:12 h L:D cycle). The parasitoid, *C. chlorideae* culture maintained for over 40 generations on *H. armigera* larvae under laboratory conditions, was used in the experiments. Males and females of similar age were kept in plastic cages (2 L capacity) for mating, and fed on 10% honey solution. For oviposition, the mated females were transferred to 50 ml Plexi glass vials placed in an inverted position on a Petri dish. Single second-instars of *H. armigera* were offered to the parasitoid females for oviposition. After parasitization, *H. armigera* larvae were placed on artificial diet for parasitoid development.

Effect of different mating regimes on progeny production in C. chlorideae—To study the influence of courtship behaviour, oviposition, and mating regimes on progeny production, the *C. chlorideae* adults were monitored for the courtship behaviour visually. Numbers of probings by same or different parasitoid females on the same *H. armigera* larva, and their effect on survival of the host larvae were recorded (data not reported). Fifteen females (0 to 5 days old) were monitored for their mating behaviour, and those mating with two-males simultaneously (referred to as two male-mated females hereafter) were collected individually along with the males, and kept in another cage (2 L capacity). Females mated with a single male were used as controls. There were three replications (one female per replication, i.e., N = 3) for each one- and two-male mated females. Only 3 females (mated with two-males out of the 15 females in a cage) were able to parasitize the *H. armigera* larvae successfully. Observations were recorded on fecundity (numbers of host larvae probed during total adult life), cocoon formation, adult emergence, and sex-ratio of the progeny.

Influence of different mating and parasitism regimes on biology and sex-ratio of C. chlorideae—The biology and sex-ratio of *C. chlorideae* reared on *H. armigera* larvae were studied under four mating schemes. The *C. chlorideae* females were allowed to mate for 72 h inside a cage (2 L capacity), except for one treatment, where the unmated females were used for parasitization. The following mating and parasitization schemes were used:

(i) Males and females kept in companionship throughout the life span, and 20 *H. armigera* larvae exposed for parasitism daily (T1).
(ii) Males removed after 72 h of mating, and the females used for parasitizing 20 *H. armigera* larvae daily (T2).
(iii) Males and females kept in companionship throughout the life span, and allowed to parasitize as many *H. armigera* larvae as possible daily (T3).
(iv) Unmated females (72 h old) used to parasitize as many *H. armigera* larvae as possible daily (T4).

All the females were allowed to parasitize *H. armigera* larvae till death. No fresh larvae were offered to the females for parasitism when a female took >10 min for egg laying. After parasitism, the *H. armigera* larvae were transferred to the artificial diet. There were five replications for each experiment (one female per replication, i.e., N = 5), in a completely randomized design. Data were recorded on longevity and fecundity of the parasitoid females, larval and pupal periods, cocoon formation, adult emergence, and sex-ratio of the progeny in each treatment.

The unmated *C. chlorideae* females produce only males. The role of males and females from mated and/or unmated females on progeny production and sex-ratio was studied in the following three cross-combinations:

(i) Progeny from unmated females (UF).
(ii) Progeny from mated females × males (designated as F_1).
(iii) F_1 females × males from unmated females (designated as BC_1F_1).

The parasitoid females of similar age were used in combinations (i) and (ii). There were four crosses for each combination (N = 4), and the observations were recorded on fecundity, cocoon formation, adult emergence, and sex ratio of the offspring in each cross.

Statistical analysis—The data were subjected to normality and homogeneity tests, and the data on percentages and insect numbers were transformed using Arcsine and square root transformation, respectively. Data were subjected to analysis of variance (ANOVA) using GenStat® 10th version statistical analysis program. Significance of differences between the treatments was judged by F-test, and the means were compared using least significant difference (LSD) at $P \leq 0.05$. The association between female longevity and fecundity was analyzed using Spearman's correlation analysis.

Results

Effect of different mating regimes and courtship behaviour on progeny production in C. chlorideae—Only 20% (3 out of 15 females observed) females mated with two-males were able to parasitize the *H. armigera* larvae, while 87% (13 out of 15 females) of the females mated only with one-male were able to parasitize the host larvae successfully. There were no significant differences between the females mated with one- or two-males in terms of fecundity (P = 0.433, F = 0.95, df = 2), cocoon formation (P = 0.163, F = 4.68, df = 2), and adult emergence (P = 0.153, F = 5.07, df = 2). However, there were significant differences in sex-ratio of the progenies of the females mated with one- or two-males (P = 0.017, F = 57.94, df = 2). Proportion of females and males obtained from the progenies of the females mated with one-male were almost equal, but the sex-ratio was male biased in case of progeny obtained from females mated with two-males (Table 1).

The *C. chlorideae* females located the *H. armigera* larvae quite rapidly (within 2 to 3 min). When a single second-instar *H. armigera* larva was offered for parasitism, the female parasitoid moved towards the larva, and started moving the antennae around the larva. This process was repeated several times till it found a suitable site for oviposition. After parasitization, the females moved away from the host larva. In the absence of another host larva in the immediate vicinity, the parasitoid female attempted to parasitize the same larva twice. Up to 7 probings were observed on the same larva at an interval of 20 to 60 seconds, but only one cocoon was recovered from each *H. armigera* larva. During multiple probings (by one or more females), survival of host larvae was very low, and sometimes the host larvae died during the parasitism process.

Influence of mating and parasitism regimes on biology and sex-ratio of C. chlorideae—Mating and parasitism regimes had no significant effect on cocoon formation (P = 0.065, F = 3.15, df 12), adult emergence (P = 0.615, F = 0.62, df = 12), larval period (P = 0.603, F = 0.64, df = 12), and pupal period (P = 0.145, F = 2.16, df = 12) of *C. chlorideae* (Table 2). However, the mating and parasitism regimes showed a significant influence on female longevity (P = 0.020, F = 4.84, df = 12), fecundity (P = 0.011, F = 6.33, df = 12), and the sex-ratio (P < 0.001, F = 191.19, df = 12). Fecundity and longevity of the females of T2 were significantly greater than the females of T1 and T4 (Table 2). There was a significant and positive correlation (r = 0.84, $P \leq 0.01$) between fecundity and longevity of the parasitoid females. The unmated females produced only males. However, the proportion of females obtained in the progeny from T1 were significantly more than the progeny from T2, while, the proportion of females in T2 and T3 were significantly on par with each other (Table 2).

Fertility status of C. chlorideae males obtained from unmated females—There were no significant differences for fecundity (P = 0.31, F = 1.44, df = 6), cocoon formation (P = 0.09, F = 3.75, df = 6), and adult emergence (P = 0.49, F = 0.81, df = 6) in progenies of *C. chlorideae* females from unmated females or the females mated with males from mated or unmated females. The sex-ratio varied significantly across different cross combinations (P < 0.001, F = 264.48, df = 6). The unmated *C. chlorideae* females produced only males, while the females mated with the males obtained either from mated or unmated females produced both females and males (Table 3). There was a significant reduction in proportion of females in the progeny derived from F_1 females × males from unmated females BC_1F_1 as compared to the progeny obtained from a normal mating system (F_1).

Table 1—Fecundity, survival, and sex ratio of the progenies from *Campoletis chlorideae* females mated with one- or two-males
[Values are mean ± SE from 3 replications. Figures in the parenthesis are [a)] square root transformed, and [b)] Arcsine transformed.]

Treatments	Fecundity female^{-1}	Cocoon formation (%)	Adult emergence (%)	Proportion females (%)
One-male mated females	262.7 ± 10.6 (16.2 ± 0.3)^{a}	81.8 ± 3.0 (64.9 ± 2.3)^{b}	56.9 ± 4.0 (49.0 ± 2.3)^{b}	48.0 ± 2.1 (43.9 ± 1.2)^{b}
Two-male mated females	205.3 ± 53.0 (14.0 ± 2.0)	72.8 ± 1.2 (58.5 ± 0.7)	45.0 ± 1.5 (42.2 ± 0.9)	29.5 ± 2.3 (32.9 ± 1.4)
LSD ($P \leq 0.05$)	NS	NS	NS	(6.22)

NS = Non-significant at $P \leq 0.05$.

Table 2—Influence of different mating regimes and numbers of *Helicoverpa armigera* larvae offered for parasitism on survival and development of *Campoletis chlorideae*

[Values are mean ± SE from 5 replications. Figures in the parenthesis are [a]square root transformed, and [b]Arcsine transformed.]

Treatments	Fecundity female^{-1}	Cocoon formation (%)	Adult emergence (%)	Larval period (days)	Pupal period (days)	Female longevity (days)	Proportion females (%)
T1	242.9 ± 28.6 (15.4 ± 1.0)[a]	76.5 ± 3.6 (61.2 ± 2.3)[b]	57.4 ± 1.4 (49.3 ± 0.8)[b]	7.4 ± 0.03	5.8 ± 0.12	14.8 ± 1.1 (3.8 ± 0.1)[a]	52.9 ± 2.6 (46.7 ± 1.5)[b]
T2	335.2 ± 11.4 (18.3 ± 0.3)	80.3 ± 1.1 (63.7 ± 0.8)	53.9 ± 0.7 (47.2 ± 0.4)	7.3 ± 0.04	5.9 ± 0.02	18.8 ± 0.4 (4.3 ± 0.04)	44.2 ± 4.2 (41.6± 2.4)
T3	308.2 ± 26.5 (17.5 ± 0.7)	76.2 ± 3.0 (61.0 ± 2.1)	55.2 ± 3.3 (48.0 ± 1.9)	7.3 ± 0.05	5.9 ± 0.02	16.0 ± 1.2 (4.0 ± 0.2)	47.0 ± 1.9 (43.3 ± 1.1)
T4	219.2 ± 17.6 (14.8 ± 0.6)	84.2 ± 1.3 (66.6 ± 1.0)	56.5 ± 1.3 (48.7 ± 0.7)	7.4 ± 0.06	6.0 ± 0.03	13.3 ± 1.1 (3.6 ± 0.2)	00.0 ± 0.0 (00.0 ± 0.0)
LSD ($P \leq 0.05$)	(2.11)	NS	NS	NS	NS	(0.42)	(4.91)

T1 = Males and females kept in companionship throughout life span, and 20 host larvae exposed for parasitism each day. T2 = Males removed after 72 h of mating and 20 host larvae exposed for parasitism per day. T3 = Males and females kept in companionship throughout life span, and host larvae exposed to maximum parasitism potential per day. T4 = Unmated females and host larvae exposed to maximum parasitism potential per day. NS = Non-significant at $P \leq 0.05$.

Table 3—Fertility status of *C. chlorideae* males obtained from unmated females (haploid males)

[Values are mean ± SE from 4 replications. Figures in the parenthesis are [a]square root transformed, and [b]Arcsine transformed.]

Treatments	Fecundity female^{-1}	Cocoon formation (%)	Adult emergence (%)	Proportion females (%)
Unmated females	247.3 ± 11.3 (15.7 ± 0.4)[a]	74.7 ± 2.5 (59.9 ± 1.7)[b]	56.7 ± 1.6 (48.9 ± 0.9)[b]	00.0 ± 0.0 (00.0 ± 0.0)[b]
F_1[¥]	268.5 ± 9.5 (16.4 ± 0.3)	78.9 ± 3.2 (62.8 ± 2.2)	57.0 ± 2.8 (49.1 ± 1.6)	47.7 ± 2.3 (43.7 ± 1.3)
BC_1F_1[§]	259.5 ± 10.8 (16.1 ± 0.3)	66.2 ± 2.8 (54.5 ± 1.7)	51.7 ± 3.7 (46.0 ± 2.1)	36.7 ± 2.8 (37.3 ± 1.7)
LSD ($P \leq 0.05$)	NS	NS	NS	(5.02)

[¥] F_1 = Standard females × standard males (females/males obtained from pooled population). [§] BC_1F_1 = F_1 females × males obtained from unmated females. NS = Non-significant at $P \leq 0.05$.

Discussion

Sex-ratio of the offspring from females that had mated with two-males was male biased, indicating a disadvantage of mating with more than one male. The loss in parasitism potential of the females mated with two-males may be due to physical distortion of ovipositor. *Campoletis sonorensis* symbiotic Polydnavirus (*CslV*) transmitted with the egg into the host larvae during parasitism has been reported to interact with the prothoracic glands to reduce ecdysone levels resulting in reduction of growth of the parasitized host, and increase the parasitoid survival[8,19,20,21]. Oviposition by *C. chlorideae* in larvae of *H. armigera* has also been reported to transmit oviduct calyx associated polydnavirus (CcIV) resulting in phenoloxidase inhibition due to suppression in expression of prophenoloxidase (proPO) or accelerate the degradation of proPO in the host hemolymph[22,23]. Low survival/recovery of the parasitoid larvae in case of multiple probings could be because of early mortality of host larvae due to lack of phenoloxidase activity and higher amounts of CcIV. Low hemolymph protein content in the host larvae has also been reported to result in delayed and abnormal development of parasitoid larvae[24].

There were no significant effects of mating and parasitism regimes on the survival and post-embryonic development of *C. chlorideae*. However, mating and parasitism regimes showed a significant effect on fecundity and female longevity. The sex-ratio governs the success/failure of any biological control program. Earlier reports indicate that the age of males and females at the time of mating, and host density can influence the sex ratio in *C. chlorideae*[10-12]. However, Comins and Wellings[25] suggested that sex-ratio in arrhenotokous parasitoids may be a function of male parasitoid population density, female parasitoid population density, host population density, and the ratio of parasitoid females to hosts. In the present studies, we did not observe a significant effect of different mating and parasitism regimes on the sex-ratio of *C. chlorideae* (except for unmated females, which produced only males). In many hymenopterans, sex is determined at a single polymorphic sex locus. The individuals that are heterozygous at this locus develop as females, whereas homozygotes develop as diploid, and hemizygotes as haploid males[26], which under inbreeding, produce more males, and accounts for single-locus complimentary sex determination mechanism[17]. The ichneumonid parasitoid, *C. chlorideae* produced fertile males in both the cases, either obtained from unmated or mated females. However, the mated braconid females produce sterile males[27,28]. A significant reduction in females was recorded in the progeny from males obtained from unmated females, suggesting the role of some nuclear factors in regulation of sex-ratio in *C. chlorideae*.

Acknowledgment

Thanks are due to Messrs V. Venkateshwara Rao and S.V.N. Chandra for technical support during the studies, and Dr. David Hoisington for valuable comments on the manuscript.

References

1 Pawar C S, Bhatnagar V S & Jadhav D R, *Heliothis* species and their natural enemies, with their potential for biological control, *Proc Indian Acad Sci, India*, 95B (1989) 695.

2 Romeis J & Shanower T G, Arthropod natural enemies of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) in India, *Biocontr Sci Tech*, 6 (1996) 481.

3 Dhillon M K & Sharma H C, Survival and development of *Campoletis chlorideae* on various insects and crops hosts: Implications for *Bt* transgenic crops, *J Appl Entomol*, 131 (2007) 179.

4 Tikar S N, Rao N G V, Satpute N S & Dandale HG, Egg laying preference and larval parasitization of *Helicoverpa armigera* (Hubner) on weed host *Lagascea mollis* (Cav) in comparison with cotton at Akola, *Insect Environ*, 6 (2001) 180.

5 Dhembare A J, Preference of host *Helicoverpa armigera* by larval parasite *Campoletis chlorideae* Uchida, *J Exp Zool*, 2 (1999) 27.

6 Sharma H C, Dhillon M K & Arora R, Effects of *Bacillus thuringiensis* δ-endotoxin-fed *Helicoverpa armigera* on the survival and development of the parasitoid *Campoletis chlorideae*, *Entomol Exp Appl*, 126 (2008) 1.

7 Pandey A K & Tripathi C P M, Effect of temperature on the development, fecundity, progeny sex ratio and life-table of *Campoletis chlorideae*, an endolarval parasitoid of the pod borer, *Helicoverpa armigera*, *Biocontr*, 53 (2008) 461.

8 Gunasena G H, Vinson S B & Williams H J, Interrelationships between growth of *Heliothis virescens* (Lepidoptera: Noctuidae) and of its parasitoid, *Campoletis chlorideae* (Hymenoptera: Ichneumonidae), *Ann Entomol Soc America*, 82 (1989) 187.

9 Dhillon M K & Sharma H C, Temperature influences the performance and effectiveness of field and laboratory strains of the ichneumonid parasitoid, *Campoletis chlorideae*, *Biocontr*, 54 (2009) 743.

10 Venkatesan T, Rao N S & Joshi S, Influence of mating age, parental sex-ratio, and host age on the female progeny emergence of *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae), *Shashpa*, 6 (1999) 145.

11 Kumar N, Kumar A & Tripathi C P M, Sex ratio of *Campoletis chlorideae* Uchida in response to *Helicoverpa armigera* (Hubner) density, *Insect Sci Appl*, 20 (2000) 73.

12 Pandey A K, Tripathi S & Tripathi C P M, Effects of parental age at mating on the fecundity and progeny sex ratio of *Campoletis chlorideae*, an endolarval parasitoid of the pod borer, *Helicoverpa armigera*, *Biocontr*, 54 (2009) 47.

13 Cook J M, Sex determination in the Hymenoptera: A review of models and evidence, *Hered*, 71 (1993) 421.

14 Butcher R D J, Whitfield W G F & Hubbard S F, Single-locus complimentary sex determination in *Diadegma chrysostictos* (Gmelin) (Hymenoptera: Ichneumonidae), *J Hered*, 91 (2000) 104.

15 Beukeboom L W & Kamping A, No patrigens required for femaleness in the haplodiploid wasp *Nasonia vitripennis*, *Genet*, 172 (2006) 981.

16 de Boer J G, Ode P J, Vet L E M, Whitfield J & Heimpel G E, Complimentary sex determination in the parasitoid wasp *Cotesia vestalis* (*C. plutellae*), *J Evol Biol*, 20 (2007) 340.

17 Ode P J & Hardy I C W, Parasitoid sex ratios and biological control, in *Behavioural ecology of insect parasitoids: from theoretical approaches to field applications*, edited by E Wajnberg, C Bernstein & J J M van Alphen, (Blackwell Publishing, Oxford, UK) 2008, 253.

18 Vinson S B, Courtship behaviour and evidence for a sex pheromone in the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae), *Environ Entomol*, 1 (1972) 409.

19 Beckage N E, Endocrine interactions between endoparasitic insects and their hosts, *Ann Rev Entomol*, 30 (1985) 371.

20 Dover B A, Davies D H & Vinson S B, Degeneration of last-instar *Heliothis virescens* prothoracic glands by *Campoletis sonorensis* polydna virus, *J Invert Pathol*, 51 (1988) 80.

21 Kroemer J A & Webb B A, Characterization of an Ikb-related ankyrin gene family in the *Campoletis sonorensis* ichnovirus (*CslV*) genome, in *Annual Meeting and Exhibition, Student Competition Display Presentations, Section B. Physiology, Biochemistry, Toxicology, and Molecular Biology*, (Entomological Society of America) (http://esa.confex.com/esa/2003/techprogram/paper_11983.htm, 2003.

22 Yin L, Wang C & Qin J, Effect of the endoparasitoid *Campoletis chlorideae* on phenoloxidase activity in *Helicoverpa armigera* hemolymph, *Chin Sci Bull*, 46 (2001) 1797.

23 Yin L, Zhang C, Qin J & Wang C, Polydnavirus of *Campoletis chlorideae*: Characterization and temporal effect on host *Helicoverpa armigera* cellular immune response, *Arch Insect Biochem Physiol*, 52 (2003) 104.

24 Ren L, Yang Y Z, Li X, Miao L, Yu Y S & Qin Q L, Impact of transgenic Cry1A plus CpTI cotton on *Helicoverpa armigera* (Lepidoptera: Noctuidae) and its two endoparasitoid wasps *Microplitis mediator* (Hymenoptera: Braconidae) and *Campoletis chlorideae* (Hymenoptera: Ichneumonidae), *Acta Entomol Sin*, 47 (2004) 1.

25 Comins H N & Wellings P W, Density-related parasitoid sex-ratio: Influence of host-parasitoid dynamics, *J Ani Ecol*, 54 (1985) 583.

26 Heimpel G E, Antolin M F & Strand M R, Diversity of sex determining alleles in *Bracon hebetor*, *Hered*, 82 (1999) 282.

27 Whiting P W, Sex determination and reproductive economy in *Habrobracon*, *Genet*, 24 (1939) 110.

28 Whiting P W, Multiple alleles in complementary sex determination in *Habrobracon*, *Genet*, 28 (1943) 365.