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OFFSPRING IN RESPONSE TO PARENTAL FEMALE DENSITIES IN THE
FRUIT FLY PARASITOID *DIACHASMIMORPHA LONGICAUDATA*
(HYMENOPTERA: BRACONIDAE: OPIINAE)

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ABSTRACT

As part of an evaluation of the braconid parasitoid *Diachasmimorpha longicaudata* (Ashmead) as a biocontrol agent of *Ceratitis capitata* (Wiedemann) in Brazil, the aims in the current study were to find the best parental ratio of females to males in the rearing cages in order to get the highest female biased offspring in the parasitoid rearing process, and to verify the parasitism efficiency on *C. capitata* according to parental female densities. Three treatments were assessed: T1 (20 females: 20 males), T2 (60 females: 20 males) and T3 (100 females: 20 males). Ten late-third instars of *C. capitata* were offered daily to each female parasitoid from the 1st to the 12th d of age. The parental female productivity, fecundity, offspring sex ratio, percentage of parasitoid emergence, and daily mortality of parental females and males at different female/male densities were evaluated. The results indicated that numbers higher than 20 parental females did not affect offspring sex ratio, overall offspring production, nor the percent parasitism. Female biased offspring occurred in all three parental female/male ratios analyzed in this study, except that predominately males developed from parasitoid eggs laid in the age interval 1-2 d post emergence. Higher parasitoid female productivity and fecundity were found at the 1:1 female/male per cage density whereas lower productivity and fecundity were recorded at the 5:1 female/male ratio. Higher female/male ratio in the parental cages increased the mortality rate of females but did not influence the number of parental male deaths. The results may facilitate advancement of an optimum mass-rearing system to aid in control of *C. capitata* in Brazil.

Key Words: biological control, Brazil, parasitism, medfly, sex ratio, progeny, *Ceratitis capitata*

RESUMEN

El presente trabajo forma parte de un programa de evaluación del parasitoide braconido *Diachasmimorpha longicaudata* (Ashmead) como agente de biocontrol de *Ceratitis capitata* (Wiedemann) en Brasil. Los objetivos del estudio fueron: (a) encontrar una proporción adecuada de hembras y machos en las jaulas de multiplicación para obtener una descendencia mayoritariamente de hembras en el proceso de cría del parasitoide, y (b) verificar la eficiencia del parasitismo en *C. capitata* según las distintas densidades de hembras madres. Por tal motivo, se realizaron tres tratamientos: T1 (20 hembras: 20 machos), T2 (60 hembras: 20 machos) y T3 (100 hembras: 20 machos). Se ofrecieron diariamente larvas del 3er estadio de *C. capitata*, en una proporción de 10 larvas por cada hembra madre, durante los primeros 12 días de vida. En cada tratamiento se registró la productividad y fecundidad de las hembras madres, tasa sexual de la descendencia, porcentaje de parasitoides emergidos y mortalidad diaria de hembras y machos padres. Los resultados mostraron que números superiores de 20 hembras madres por jaula de multiplicación no afectaron la tasa sexual de la descendencia, la producción total de hijos, ni el porcentaje de parasitismo. En general, la descendencia tuvo predominancia de hembras en las tres proporciones hembras/machos padres estudiadas, con la excepción en las hembras madres de 1-2 días de edad, las cuales produjeron mayoritariamente machos. La

mayor fecundidad y productividad de hijas hembras se registró a una densidad de una hembra por macho por jaula de multiplicación, mientras que la productividad y fecundidad más baja se manifestó a una densidad de cinco hembras por macho. Además, la tasa de mortalidad de hembras madres se incrementó en las jaulas de multiplicación con altas densidades hembra/macho. Los resultados del presente trabajo pueden contribuir a la optimización de la cría masiva de *D. longicaudata* en Brasil utilizando como huésped a *C. capitata*.

Translation provided by the authors.

The major natural enemies of tephritid fruit flies in Brazil are members of the families Braconidae, Figitidae, and Pteromalidae (Hymenoptera) (García & Corseuil 2004). Parasitoid mass rearing, required for biocontrol via augmentative release, must be directed to optimize production of females rather than males (Carey et al. 1988). A higher female-biased sex ratio can be more efficient in applied biocontrol efforts with parasitoids because females can eliminate the target pest by oviposition and/or by feeding on host fluids themselves (King 2002). Therefore, understanding the different factors affecting offspring sex ratio of the parasitoid species to be mass reared is essential to get efficient parasitoid production (Wong & Ramadan 1992; Heimpel & Lundgren 2000).

In haplodiploid insects such as Hymenoptera, males develop from an unfertilized haploid egg, but females originate from a fertilized diploid egg. Offspring sex-ratio control is primarily maternal (Godfray 1994), but several biotic and abiotic factors can affect parasitoid progeny yield and offspring sex ratio (King 1987; Heimpel & Lundgren 2000). For example, the following factors have been identified in the Braconidae: host size, stage and age (Jarosik et al. 2003; Zenil et al. 2004), host species (Messing & Ramadan 2000; Quimio & Walter 2001), host plant (Jackson et al. 1996), host density (Harris & Bautista 1996), host contagion with pathogens (Orr et al. 1994), parental age (Rungrojwanich & Walter 2000), density and sex ratio of parents (Lozano et al. 1997), dispersion behavior (Paranhos et al. 2007), superparasitism (González et al. 2007), inbreeding (Stouthamer et al. 1992; Salin et al. 2004), local mate competition (Nishimura & Jahn 1996), multiple matings, postmating rest period, temperature in mating chambers, and storage temperatures for parents (Fuester et al. 2003).

Several species of braconid parasitoids belonging to the subfamily Opiinae have been mass-reared and augmentatively released against economically important tephritid fruit fly species in tropical and subtropical countries (Purcell 1998; Rendon et al. 2006). One such braconid is *Dia-chasmimorpha longicaudata* (Ashmead), a koinobiont larval-pupal endoparasitoid associated with numerous species of tephritid fruit flies (Wharton & Gilstrap 1983; Ovruski et al. 2000). This parasitoid species is currently considered to be one of

the most significant biological control agents for augmentative release programs against fruit fly pests in Latin American (González et al. 2007).

The Mediterranean fruit fly and several *Anastrepha* species are major pests of fruit crops worldwide and in Brazil. Parasitoid species were first reared on a *C. capitata* bisexual strain (Walder et al. 1995) in Brazil. Currently, in the Moscamed facility in Brazil the viability for mass-rearing *D. longicaudata* on larvae of a genetic sexing (males-only) strain of a temperature sensitive lethal (*tsl*) *C. capitata* strain has been studied as part of an augmentative release program against *C. capitata* (Wiedemann) in the tropical fruit growing areas of northern Brazil (Lopes & Paranhos 2006; Paranhos 2007; Malavasi et al. 2007).

The objectives in the current study were to find the best parental ratio of females to males of *D. longicaudata* in rearing cages in order to get the highest female biased offspring in the rearing of *D. longicaudata*, and to verify the parasitism efficiency on *C. capitata* according to parental female densities.

MATERIAL AND METHODS

Study Site and Biological Material

The study was conducted in the Laboratory of Radio-Entomology at CENA/University of São Paulo located in Piracicaba Town, São Paulo State, Brazil, in 1998. Laboratory conditions were 25-27°C, 60-85% RH, with a photoperiod of 12:12 h (L:D). The colony of *D. longicaudata* used in the experiments was raised and maintained on late-third instars of the bisexual strain of *C. capitata* in the laboratory according to rearing methods described by Walder (2002).

Experimental Procedure

Adults of *D. longicaudata* were kept in aluminum-framed, mesh-covered experimental cages (30 × 30 × 30cm) provided with agar-agar (10 g), water (700 mL) and bee honey (300 mL). Males, which emerge before females, were collected and maintained inside experimental cages. After the second day, virgin females were collected just after emerging and also maintained in cages.

Late-third instars of *C. capitata* (7 d old) from a wild bisexual strain were separated from arti-

ficial diet, irradiated at 60 Gy dose (Co^{60} source, γ rays) to avoid the emergence of flies, and used as hosts for all experiments. The irradiated naked larvae were placed in cylindrical PVC dishes (5 cm diameter, 1 cm height) that had the bottom side covered with organdy mesh. These oviposition units were exposed on the top screen of experimental cages for 40 min to female parasitoids. Ten irradiated late third instars of *C. capitata* in the oviposition units described above were offered daily to each female parasitoid from the 1st to 12th d of age. Three proportions of female/male parasitoids were tested independently: 20 females and 20 males (1:1 female/male proportion), 60 females and 20 males (3:1), and 100 females and 20 males (5:1). Host larval density per female parasitoid was the same, always 10 larvae/female. Thus, in total 200, 600, and 1000 host larvae were offered per day and per experimental cage at the female densities noted above of 20, 60, and 100 female, respectively. The number of host larvae provided to surviving parasitoids was proportionally decreased as parental females died during the 12-day oviposition period. For each reared batch of parasitoids, 10 replicate experimental cages were used for each female/male parasitoid density, and each treatment was repeated 3 times with fresh parasitoids and host larvae. The cages were positioned at random on a table and kept under the laboratory conditions described above.

After exposure to the parasitoids, the fruit fly larvae were carefully transferred to plastic cups (250 mL) containing vermiculite as the pupation medium, and perforated lids to allow gas exchange. One cup was used for each treatment, maternal parasitoid age, and replicate. The cups containing pupae were kept under the laboratory conditions described above until the emergence of adult parasitoids. The number of male and female parasitoids emerged was recorded daily.

The following parameters were evaluated: (1) parental female productivity, calculated as the average number of live adult offspring (males + females) produced by a parental female (Bautista et al. 1998); (2) fecundity, estimated as the average of female offspring by parental females at age, (m_x) (Carey 1993); (3) offspring sex ratio, calculated as the number of female offspring divided by the sum of female and male offspring produced by surviving females at age x multiplied by 100; (4) percentage of parasitoid emergence, calculated as the number of adult parasitoids emerged divided by the total number of exposed host larvae; and (5) daily mortality of parental females and males at different female/male densities. Data on adult offspring, percent parasitism, and parental mortality rate were pooled at 2-day maternal age intervals.

Data Analysis

A completely randomized design was used. A two-way analysis of variance (ANOVA) with treatment and the maternal age interval as grouping factors was used to analyze the difference in parental female productivity and fecundity, offspring sex ratio, and percentage of adult parasitoid eclosion. Mean comparisons were analyzed by Tukey's honestly significant difference (HSD) test at $P = 0.05$. An arcsine square root transformation was applied to the percentage data to normalize their distribution. However, original means (\pm SEM) are used for data presentation. A Kruskal-Wallis one way analysis of variance on ranks and Median comparisons test ($P = 0.05$) was performed to analyze the difference in the number of parental female and male deaths among treatments and maternal age intervals, because normality and homoscedasticity assumptions were not met and data transformations were unsuccessful.

RESULTS

Overall, the mean live progeny produced by a female during 12 d of life at 1:1 female/male rate was 2.7-, and 4.7- times higher than in high parental female density treatments ($F_{2,100} = 170.45$, $P < 0.0001$) (Table 1). A similar trend was recorded for mean fecundity yield by females throughout the 12 d of a test ($F_{2,100} = 133.11$, $P < 0.0001$ (Table 1). The overall progeny production (mean \pm SEM) was not different among the 3 parasitoid female/male densities (711.3 \pm 33.6, 791.7 \pm 4.8, and 750.3 \pm 11.9 parasitoids at 1:1, 3:1, and 5:1 female/male densities, respectively) ($F_{2,6} = 3.75$, $P = 0.0877$). In all 3 female/male ratios evaluated during the 12-d period, both the cumulative fecundity and progeny produced every 2 d increased significantly after the maternal age interval 1-2 d post eclosion, and did not increase or decrease significantly after parental female age 5-6 d ($F_{5,100} = 20.48$, $P < 0.0001$ and $F_{5,100} = 18.32$, $P < 0.0001$, respectively) (Table 1). Overall, the mean sex ratio of offspring was not significantly different for all three treatments ($F_{2,100} = 0.38$, $P = 0.6865$) (Table 2). Approximately 70% of the total progeny produced were females during the 12-d period at 1:1, 3:1, and 5:1 female/male densities. A male-biased sex ratio was recorded at maternal age interval 1-2 d. Thereafter, offspring sex ratio was female-biased and the female proportion, ranging between 70 and 86%, did not increase or decrease significantly (Table 2).

There was a difference ($F_{2,100} = 206.80$, $P < 0.0001$) in the mean percentage of parasitoid emergence when compared among the 1:1 female/male density and the highest parental female density treatments (Table 2). Moreover, the analysis of parasitoid emergence showed a highly sig-

TABLE 1. OFFSPRING PRODUCTION OF *DIACHASMIMORPHA LONGICAUDATA* REARED ON *CERATITIS CAPITATA* IRRADIATED LARVAE AT DIFFERENT MATERNAL AGE INTERVALS AND PARASITOID FEMALE/MALE DENSITIES

Maternal age intervals (days)	Female productivity (mean ± SEM) ^a			Fecundity (mean ± SEM) ^a		
	Female/male densities			Female/male densities		
	1:1	3:1	5:1	1:1	3:1	5:1
1-2	0.07 ± 0.04 a	0.04 ± 0.01 a	0.02 ± 0.01 a	0.03 ± 0.02 a	0.02 ± 0.01 a	0.01 ± 0.01 a
3-4	0.26 ± 0.03 b	0.11 ± 0.01 ab	0.06 ± 0.01 b	0.18 ± 0.03 b	0.07 ± 0.01 ab	0.05 ± 0.01 b
5-6	0.35 ± 0.03 c	0.13 ± 0.02 b	0.07 ± 0.01 b	0.28 ± 0.02 c	0.11 ± 0.01 b	0.06 ± 0.01 b
7-8	0.35 ± 0.01 c	0.14 ± 0.01 b	0.08 ± 0.01 b	0.28 ± 0.01 c	0.12 ± 0.01 b	0.07 ± 0.01 b
9-10	0.38 ± 0.02 c	0.13 ± 0.01 b	0.07 ± 0.01 b	0.30 ± 0.02 c	0.10 ± 0.01 b	0.05 ± 0.01 b
11-12	0.37 ± 0.02 c	0.11 ± 0.01 b	0.07 ± 0.01 b	0.29 ± 0.02 c	0.09 ± 0.01 b	0.05 ± 0.01 b
Overall mean ^b	0.32 ± 0.02 A	0.12 ± 0.01 B	0.07 ± 0.01 C	0.23 ± 0.02 A	0.09 ± 0.08 B	0.05 ± 0.01 C

^aMeans within a column followed by the same small letter do not differ significantly (Tukey's test, $P < 0.05$).

^bMeans in the same row followed by a different capital letter differ significantly (Tukey's test, $P < 0.05$).

nificant difference between parental female age intervals ($F_{5,100} = 40.21, P < 0.0001$). In all treatments, the parasitoid emergence produced by 1-2-d-old females was significantly lower than in older maternal ages (Tukey test, $P < 0.05$) (Table 2). The highest percentage of parasitoid emergence was recorded for 7-8-d-old females in 1:1, 3:1 and 5:1 female/male ratios, although there was no significant difference (Tukey test, $P < 0.05$) with lower maternal age intervals (3-4 and 5-6 d old) nor with higher maternal age intervals (9-10 and 11-12 d old) (Table 2).

There was a significant difference ($H(2, n = 108) = 17.95, P < 0.0001$) in the total number of parental female deaths at end of the 12-d period between the different female/male densities. Approximately, 3 and 12 times as many females died at the 3:1 and 5:1 female/male densities, respectively, as compared to the 1:1 female/male density

(0.06 ± 0.04 females). Thus, there was no significant difference between the observed and expected number of female deaths at the 3:1 female/male density, but a greater number of females died at the 5:1 female/male ratio than would be expected ($\chi^2 = 16.71, df = 2, P = 0.0002$). On the contrary, changes in female/male densities did not significantly influence the total number of parental male deaths ($H(2, n = 108) = 2.14, P = 0.3428$). The number of males dead at the 3:1 and 5:1 female/male densities only was 1.3 and 2.3 times the number occurring at the 1:1 female/male density (0.22 ± 0.09 females, mean ± SEM), respectively. There was no significant difference in the number of female deaths among maternal age intervals at the 1:1, 3:1 and 5:1 female/male densities ($H(5, n = 36) = 4.12, P = 0.35326; H(5, n = 36) = 4.67, P = 0.4579; H(5, n = 36) = 11.5, P = 0.0574$, respectively).

TABLE 2. OFFSPRING SEXUAL RATE AND PERCENTAGE OF PARASITOID EMERGENCE AT DIFFERENT *D. LONGICAUDATA* FEMALE AGE INTERVALS AND PARASITOID FEMALE/MALE PROPORTIONS.

Maternal age intervals (days)	Sex Ratio (% female progeny) (mean ± SEM) ^a			Parasitoid Emergence (%) (mean ± SEM) ^a		
	Female/male densities			Female/male densities		
	1:1	3:1	5:1	1:1	3:1	5:1
1-2	24.45 ± 11.45 a	27.53 ± 12.46 a	30.06 ± 13.59 a	0.72 ± 0.37 a	0.38 ± 0.17 a	0.23 ± 0.10 a
3-4	69.99 ± 3.02 b	70.01 ± 2.83 b	70.50 ± 4.62 b	2.58 ± 0.33 b	0.95 ± 0.05 ab	0.62 ± 0.05 b
5-6	79.64 ± 2.67 b	82.70 ± 2.91 b	82.22 ± 2.67 b	3.48 ± 0.29 b	1.08 ± 0.02 b	0.71 ± 0.05 b
7-8	77.83 ± 2.96 b	85.86 ± 2.63 b	85.25 ± 1.73 b	3.78 ± 0.12 b	1.15 ± 0.01 b	0.83 ± 0.03 b
9-10	79.17 ± 3.21 b	78.97 ± 3.27 b	76.82 ± 2.74 b	3.56 ± 0.16 b	1.07 ± 0.03 b	0.70 ± 0.06 b
11-12	78.67 ± 2.86 b	74.70 ± 3.62 b	79.21 ± 3.25 b	3.68 ± 0.21 b	0.98 ± 0.08 b	0.66 ± 0.17 b
Overall mean ^b	68.28 ± 8.89 A	69.94 ± 8.79 A	70.68 ± 8.37 A	2.97 ± 0.48 A	0.94 ± 0.11 B	0.63 ± 0.09 B

^aMeans within a column followed by the same letter do not differ significantly (Tukey's test, $P < 0.05$).

^bMeans in the same row followed by a different capital letter differ significantly (Tukey's test, $P < 0.05$).

DISCUSSION

Our results indicated that providing the experimental rearing cages with higher numbers than 20 parental females did not affect the sex ratio of their offspring nor did it result in significantly greater progeny production, or fecundity. Female biased progeny resulted in all tests from parasitoid females older than 2 d in agreement with observations by Ashley & Chambers (1979) on *D. longicaudata* parasitizing *Anastrepha suspensa* (Loew). These authors worked with 3 different parental parasitoid densities in the rearing cages but a 1:1 female/male ratio was always maintained. On the other hand, Ramadan et al. (1994) indicated that the female offspring of *Fopius arisanus* (Sonan), an opiine egg parasitoid of tephritid flies, was influenced by changes in the parental parasitoid density when eggs of *Bactrocera dorsalis* (Hendel) were exposed to different parasitoid female/cage densities. However, these authors pointed out that the offspring sex ratios always favored males. With exception of recorded data by Baeza Larios et al. (2002), female-biased sex ratios in populations of *D. longicaudata* have been recorded in the field on *Bactrocera correcta* (Bezzi) in Central Thailand (Kitthawee 2000), under conditions of mass-rearing on *B. dorsalis* (Wong & Ramadan 1992; Wong 1993; Messing et al. 1993; Purcell et al. 1994; Vargas et al. 2002) and on *Anastrepha ludens* (Loew) (Cancino et al. 2002a,b; Montoya et al. 2000a,b; Cancino & Montoya 2004; González et al. 2007), and from laboratory rearing on *A. suspensa* (Greany et al. 1976; Ashley & Chambers 1979), on *Bactrocera papayae* Drew & Hancock (Petcharat & Petcharat 1997), on *Anastrepha obliqua* (Macquart) and *A. ludens* (Eben et al. 2000), on *C. capitata* (Ovruski et al. 2003), on both wild and genetic sexing strains of *C. capitata* (Viscarret et al. 2006), and on *Anastrepha fraterculus* (Wiedemann) (Ovruski et al. 2007).

In contrast to our data, Viscarret et al. (2006) found no influence of *D. longicaudata* female age on sex ratio of progeny obtained from *C. capitata* puparia. It is possible that the difference between the data of Viscarret's et al. (2006) and our data might be due to the distinct preoviposition and oviposition periods of the *D. longicaudata* females recorded, as was noted by Avilla & Albajes (1984), in the opiine *P. concolor* reared on *C. capitata* and *Bactrocera oleae* Gmél. During the preoviposition period, *D. longicaudata* females tend to mate and to mature ovarian eggs (Wong & Ramadan 1992). Nevertheless, emerged females during the first 24 h have been recorded ovipositing, but with few offspring resulting (Greany et al. 1976), mainly males (Ashley & Chambers 1979). Viscarret et al. (2006) reported preoviposition and oviposition periods of approximately 3-4 d and 23-28 d, respectively, while we only recorded offspring sex ratios

throughout the 12-d oviposition period. In general, short oviposition periods for *D. longicaudata*, which ranged between 9 and 16 d, have been reported in the literature (Ashley & Chambers 1979; Wong & Ramadan 1992; Vargas et al. 2002). Changes in the offspring sex ratios as the parental female aged have been reported in other opiine larval parasitoid species, such as *P. fletcheri* (Wong & Ramadan 1992; Wong 1993), *Diachasmimorpha tryoni* (Cameron) (Ramadan et al. 1989a,b; Wong et al. 1990; Wong & Ramadan 1992), and in the opiine egg parasitoid *F. arisanus* (Ramadan et al. 1994; Bautista et al. 1998). In general, newly emerged females as well as older females produce male-biased sex ratios (Wong et al. 1990).

The offspring sex ratio biased toward females in *D. longicaudata* found in the present study may reflect the fertilization of parasitoid eggs as a result of successful mating irrespective of the parental female/male ratio; or/and may indicate differential survival of the sexes during parasitoid post-embryonic development; or/and may reveal a greater number of female eggs deposited in the presence of conspecifics. These hypotheses have been documented as probable factors influencing offspring sex ratios (King 1993; Godfray 1994; Heimpel & Lundgren 2000). Other possible explanations could involve genetic mechanisms such as the presence of high sex allele diversity within the laboratory culture of *D. longicaudata*, as was documented by Heimpel & Strand (1999) and Antolin et al. (2003) for a commercial mass rearing culture of the braconid, *Habrobracon hebetor* Say. Recently, superparasitism has also been proposed to explain the presence of female biased sex ratio in *D. longicaudata*, especially at high parasitoid female/rearing cage densities (González et al. 2007).

Our results showed that more *D. longicaudata* females inside rearing cages without varying the parasitoid female of *C. capitata* larvae ratio did not significantly increase the overall offspring production, nor was the percentage of adult parasitoid emergence favored. Moreover, we showed that the highest parasitoid female productivity and fecundity were found at 1:1 female/male per cage density whereas the lowest productivity and fecundity were recorded at 5:1 female/male ratio. These results could be attributed to negative effects of overcrowding parental females inside experimental rearing cages at the upper parasitoid densities, such as: (1) a higher prevalence of aggressive local competition among females for sites on the parasitization unit (Ashley & Chambers 1979; Ramadan et al. 1994), which can affect their habitual oviposition behavior (Lawrence 1978); (2) a significantly greater number of parental female deaths (Ashley & Chambers 1979); and (3) a extremely higher host larvae mortality produced by the parasitoid females (Montoya et

al. 2000b; González et al. 2007). Our data and personal observations correspond well with both the first and second assumptions. Although in the current study the percentage of host larval mortality did not occur because irradiated *C. capitata* larvae were used as hosts for parasitoids, our results on parasitoid emergence suggest, in agreement with Montoya et al. (2000b) and also with the previous Ashley & Chambers' (1979) data, that a low parasitoid female/cage density should be maintained to optimize the production of *D. longicaudata*.

In conclusion, the results of the current study on progeny production efficiency of *D. longicaudata* females may facilitate the advancement of an optimum mass-rearing system using *C. capitata* for this parasitoid species in Brazil. Nonetheless, as previously recommended by Montoya et al. (2000b) and more recently by González et al. (2007), additional improvements in mass rearing process of *D. longicaudata*, particularly directed at the assessment of the parasitoid/host larvae proportion, host exposure time, and size of host exposure area, are needed to get an efficient large scale production of this fruit fly parasitoid. Thus, the host exposure period should be mainly chosen according to the parasitoid female/host ratio, which is correlated with the parental female age (Ramadan et al. 1989b). Furthermore, the quality of host larvae (e.g. size, age, and instars) must be cautiously controlled to achieve successful laboratory or factory mass rearing of *D. longicaudata* as pointed out by Wong & Ramadan (1992).

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