Biology and rearing of the fruit fly parasitoid *Biosteres arisanus*: clues to insectary propagation

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Abstract

Aspects of *Biosteres arisanus* (Sonan) (= *Opius oophilus Fullaway*) (Hymenoptera: Braconidae) development on the oriental fruit fly, *Bactrocera* (= *Dacus*) *dorsalis* (Hendel), were investigated to facilitate mass production in the insectary. Life table statistics were generated for cohorts of *B. arisanus* females. Overlap in the emergence of fruit flies and parasitoids necessitated a procedure for segregation, preferably before adult eclosion. Rate of parasitization by *B. arisanus* increased with host clutch size reaching a plateau at 20:1 host egg to female parasitoid ratio. Duration of the oviposition period influenced the level of host parasitization; host eggs were exposed to parasitoids for 24 h with minimal superparasitism. Females were highly productive within 3 weeks after emergence producing 40–70% females in the progeny. Adult males were shorter lived than females by $\approx$5 days. Based on a net reproductive rate (R_0) of >16 daughters per female parent, a population increase of 10% was predicted each day. Handling procedures that could facilitate efficient production of parasitoids are discussed.

Introduction

Tephritid fruit flies are polyphagous pests of a wide variety of fruits and vegetables (Harris, 1989). In the United States and elsewhere, malathion-based protein bait sprays are commonly used to suppress incipient outbreaks of fruit flies (Harris, 1989). However, persistent public outcries against the ecological effects of chemical pesticides on the environment and to human health have invigorated the search for alternative fruit fly control strategies.

The use of natural enemies in combination with other compatible methods, i.e., sterile insect release, presents a sound and viable option in fruit fly pest management (Knipling, 1992). In the early 1940s, braconid parasitoids were introduced into Hawaii for classical biological control of fruit flies (Back & Pemberton, 1918; Bess, 1953; Bess et al., 1961). Several species, including *Biosteres arisanus* (Sonan), were successfully established in the major island chain resulting in subsequent reductions of fruit fly populations (Bess, 1953; Bess et al., 1961; Wong & Ramadan, 1987; Vargas et al., 1993). Initially mistaken for *Biosteres persulcatus* Silvestri, *B. arisanus* was first reported in 1949 in Waikane, Oahu, from a collection of *Bactrocera dorsalis* puparia that developed as larvae in common guava, *Psidium guajava* L. (van den Bosch & Haramoto, 1951).

Biosteres arisanus is an endoparasitoid that oviposits in fruit fly eggs and 1st instar larvae. *Biosteres arisanus* readily attacks the eggs of four tephritid fruit fly species in Hawaii (Harris & Bautista, 1996; Nishida & Haramoto, 1953), but the suitability of the host for parasitoid development varies with species (Harris et al., 1991; Ramadan et al., 1992; Harris & Bautista, 1996). Occasionally, a female parasitoid oviposits more than one egg within a host (Kaya & Nishida, 1968), but being solitary, only one adult will develop and emerge from a host puparium, usually a few days after eclosion of fruit flies from unparasitized puparia (Haramoto, 1953).

Biosteres arisanus is the only known opiine egg parasitoid of tephritid flies in the Western Hemisphere (Wharton & Gilstrap, 1983). Its competitive advantage over other parasitoids when they occur in multiparasitized hosts (Bautista & Harris, 1997; van den Bosch & Haramoto, 1953), as well as resilience to adapt effectively in diverse habitats, accounted for much of its success and predominance in the Hawaiian agroecosystem (Harris et al., 1988; Wong et al., 1984; Vargas et al., 1993). These unique attributes make *Biosteres arisanus* a potential biocontrol agent against fruit flies.

Biosteres arisanus is a haplo-diploid hymenopterous endoparasitoid that would only produce female offsprings from fertilized eggs (Flanders, 1956). Despite some difficulty during earlier attempts to rear *B. arisanus* indoors (Haramoto, 1953; Chong, 1962), the parasitoid was subsequently colonized in captivity in 1989 (Harris & Okamoto, 1991). Nevertheless, the potential of *B. arisanus* for augmentative biocontrol of tephritid fruit flies necessitated biological information that could facilitate development of a mass-rearing methodology to sustain production of parasitoids for research and field releases.

In this study, we determined the duration of preimago development (number of days from egg laying to emergence of adults) and pattern of adult emergence between parasitoid and host fruit fly, effects of host egg density (clutch size) and duration of host exposure to female parasitoids on rate of fruit fly parasitization, effects of maternal age on female productivity and progeny sex ratio, and adult longevity. Life table statistics were generated for cohorts of female parasitoids.

Materials and methods

The parasitoids used in our assays were raised and maintained in the laboratory according to rearing methods by Harris & Okamoto (1991). Host eggs were obtained from colonized oriental fruit fly produced on a semi-synthetic diet formulation (Tanaka et al., 1969). Tests were conducted under laboratory conditions with ambient temperatures of 22–24 °C, and relative humidity of 60–70%. Except when otherwise indicated, photoperiod was maintained at L10:D14. Parasitoids were provided with spun honey (Sioux Honey, Sioux city, IA) and water.

Development and pattern of emergence of parasitoid and host fruit fly. Ripe papaya, *Carica papaya* L. cv. 'solo', was trimmed into 8 × 4 × 1-cm sections. The fruit rind was perforated with 10 holes (4–5 mm deep), 5 to a row, with the blunt end of a camel hair brush. A cohort of 100 fruit fly eggs (2–4 h old) was inserted into each hole with the moistened tip of the brush for a total of 1000 eggs in each fruit section. Inoculated fruit was exposed for 24 h to 50 pairs of *B. arisanus* (15–16 days old post eclosion) in a 26 × 28 × 26-cm screened cage. Subsequently, fruit was retrieved and processed as described by Harris & Bautista (1996). Nine to 10 days later, pupae were screened from vermiculite (pupation medium) (Strong-lite, Pine Butt, AR) with the use of a mesh sieve (1 mm^2). A cohort of $\approx$10 ml of pupae [mean $\pm$ (SEM) pupal count = 390 $\pm$ 8], that consisted of parasitized and unparasitized puparia was sampled, partitioned into 3 lots, and placed in separate holding containers. Each container consisted of two plastic cups (6.5 cm-diam), one inverted over the mouth of the other and secured with masking tape (3.8 cm wide). The inside of the top cup was coated with a thin film of Tanglefoot® (Grand Rapids, MI) to trap newly eclosed insects and facilitate counts of emerged parasitoids and flies.

The number of adult parasitoids (males and females) and fruit flies that emerged daily was recorded and expressed as percentage of total number of eclosed puparia. Tests were repeated 3 times using fresh batches of host puparia.

Effects of host clutch size and duration of exposure to parasitoids on fruit fly parasitization. Clutches of 25, 50, 75, 100, 125 and 150 fruit fly eggs (2–4 h old) were inoculated separately in sectioned fruits to obtain host egg to female parasitoid ratios of 5:1, 10:1, 15:1, 20:1, 25:1 and 30:1, respectively. Equal number of eggs was inserted in each of the 10 holes in the fruit. Where host eggs could not be apportioned equally per hole, extra eggs were inserted singly in any of the 10 holes at random.

Fruits inoculated with different clutch sizes were exposed separately to a cohort of five gravid females (15–16 days old post eclosion) inside a cage (26 × 28 × 26 cm) at time intervals of 4, 6, or 24 h. Thereafter, fruits were retrieved and eggs were recovered with the tip of a camel's hair brush moistened with water. Eggs were arranged in single pile on a piece of moist blotting paper, then dissected and examined individually under a stereoscope for parasitoid eggs.

Host eggs that contained 1 and 2 or more parasitoid eggs were recorded.

Tests were repeated four times. Effects of host clutch size (= 6 levels) and duration of host exposure (= 3 levels) as main factors of fruit fly parasitization were tested with a 2-way ANOVA. Mean parasitized eggs were transformed to square root $X + 1$ for homogeneity of variances before analysis of data. Untransformed values were used in the presentation of results.

Female productivity, progeny sex ratio, adult longevity and life table statistics. Twenty-five pairs of newly-emerged parasitoids were combined in a cage (26 × 28 × 26 cm). Twenty-four hours after emergence of female parasitoids and daily thereafter for ≈5 weeks, a sectioned papaya fruit inoculated with 500 eggs (2–4 h old), as described in the preceding section, was exposed to parasitoids for 24 h. Lighting was continuous during exposure of fruit fly eggs to maximize parasitization of hosts. Subsequently, host samples were processed until emergence of parasitoids (Harris & Bautista, 1996). The test was repeated four times with fresh batches of hosts and parasitoids.

Female productivity was measured by the number and sex ratio of live adult progeny recovered. Progeny yield was pooled at 5-day intervals and calculated on per female and per female per day basis. The cumulative number of female progeny was expressed as proportion of total progeny produced by a female every 5 days. A 1-way ANOVA was used to analyze differences in mean female productivity and percentage of females in the progeny among 8 levels of maternal ages. Mean separation was by Tukeys honestly significant difference (HSD) method at $P = 0.05$. Untransformed data were used in the presentation of results.

Daily mortality of adult parasitoids was concurrently recorded. Data were presented as mean percentage of male and female survivors based on 25 pairs of parasitoids used at the start of the test.

Basic life table statistics for cohorts of *B. arisanus* females were generated by the methods of Deevey (1947), Birch (1948) and Krebs (1972). Parameter estimates were calculated using data from daily records of fecundity and mortality cohorts of adult females generated in above tests. Calculations of net and daily reproductive rates were based on an average adult recovery of 64% obtained by Vargas et al. (unpubl.). In this study, cohort survival of parasitoid imma-
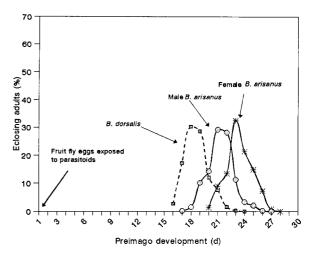

Figure 1. Duration of preimago development and pattern in adult emergence of parasitoid and host fruit fly.

tures was observed in each stage of development until emergence of adults.

Results

Development and pattern of emergence of parasitoid and host fruit fly. The emergence of fruit flies from the cohort of unparasitized pupae overlapped with those of parasitoids (Figure 1). *Bactrocera dorsalis* (total $n = 383$) developed within 16–23 days with ≈89% of fruit flies in the sample cohort completing emergence by the 20th day. Male parasitoids commenced emergence 1 day after the onset of fly emergence. Within 3 days after flies began to emerge, one-third (32% males and 5% females) of parasitoids (total $n = 2, 247$) had eclosed.

The preimago development (number of days from egg laying to adult emergence) of male *B. arisanus* was ≈2 days shorter than that of females (Figure 1). Males developed from 17–26 days with a mean of 21.1 ± 1.5 days. The peak in emergence occurred 5–6 days from initial eclosion with ≈83% of the males emerging within this 2-day period alone. Female parasitoids completed development in 20–27 days (22.9 ± 1.6 days) with peak in emergence occurring 4–5 days after initial eclosion.

Effects of host clutch size and duration of exposure to parasitoids on fruit fly parasitization. The main treatment effects, host clutch size (F=29.4; df=5,71; P<0.0001) and duration of exposure to parasitoids (F=8.4; df=2,71; P<0.001), accounted largely for

Table 1. Progeny production of *B. arisanus* at different maternal ages. Within a column, means followed by same letter are not significantly different by Tukey's test (P>0.05)

Maternal ages (Intervals in days)	Mean (± SEM) live progeny/female[a]	Mean (± SEM) live progeny/female/day
1–5	22.9 ± 1.3a	4.6 ± 0.3a
6–10	26.1 ± 4.4a	5.2 ± 0.9a
11–15	28.3 ± 0.5a	5.7 ± 0.1a
16–20	30.4 ± 4.0a	6.1 ± 0.8a
21–25	15.9 ± 7.1ab	3.5 ± 1.4ab
26–30	6.5 ± 2.3b	1.3 ± 0.5b
31–35	3.4 ± 2.0b	0.7 ± 0.4b
36–40	1.2 ± 0.7b	0.2 ± 0.1b

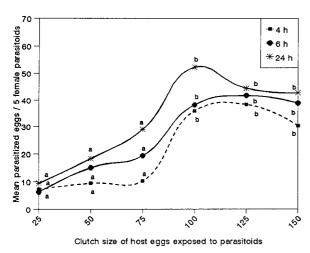[a]Values are cumulative progeny produced by a female.

Figure 2. Effects of host clutch size and duration of exposure to 5 *B. arisanus* females on fruit fly parasitization. Within each exposure time, data points with same letter are not significantly different by Tukeys test (P>0.05).

differences in mean number of parasitized fruit fly eggs (Figure 2). There was no interaction between host clutch size and duration of exposure to parasitoids (F=0.50; df=10, 71; P=0.88).

Regardless of the duration in host exposure, the oviposition response exhibited by female *B. arisanus* to varying clutch sizes of fruit fly eggs was consistently asymptotic (Figure 2). The mean number of fruit fly eggs parasitized by a cohort of five female parasitoids increased with host density, plateaued when a clutch size of 100 eggs was exposed, and did not increase or decrease significantly thereafter. The plateau at exposure times of 4, 6, and 24 h corresponded to a mean of 36 ± 6.9, 38.3 ± 4.8, and 52.3 ± 4

mean parasitized fruit fly eggs per five females, respectively. There was a 2 to 3-fold increase in mean parasitized eggs when host clutch was increased from 75 to 100 eggs. Likewise, more eggs were parasitized when exposure of host to parasitoids was prolonged. Compared with host eggs that were exposed to parasitoids for only 4 or 6 h, there was an overall gain of ≈1.5 times in the mean number of parasitized eggs when hosts were exposed for 24 h.

Female productivity, progeny sex ratio, adult longevity and life table statistics. Within 24 h after emergence, a cohort of 25 females produced a mean progeny of 54.8 (range = 19–71) or 2.2 progeny per female (Table 1). The cumulative progeny yield every 5 days reached a maximum of 30 per female (range = 23–42) at maternal ages of 16–20 days post eclosion. Thereafter, female productivity declined (F=13.1; df=8,27; P<0.0001). The rate of production (progeny per female per day) followed a similar trend, with the highest daily yield of 6 progeny per female during maternal age interval of 16–20 days post eclosion (F=13.9; df=8,27; P<0.0001). Overall, the mean progeny produced by a female in her lifetime was 134.8 ± 20.5. The progeny sex ratio favored the females (57–70%) during the first 2 weeks of maternal reproductive period (Figure 3) but became predominantly males from maternal age interval 16–20 days post eclosion and thereafter.

The survivorship data of *B. arisanus* adults concurred closely with those reported by Ramadan et al. (1992) for wild *B. arisanus*. The average life span of the males was shorter than that of females, with mean longevity of 15 ± 2.1 days (range = 1–38 days) and
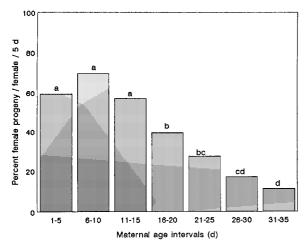
Figure 3. Influence of maternal ages on female progeny sex ratio. Bars topped with same letter are not significantly different by Tukeys test (P>0.05).

Table 2. Life table statistics of *B. arisanus* at ambient temperature of 22–24 °C and 60–70% r.h.

Demographic parameter	Calculation	Value
Gross reproductive rate, M_x	Σm_x	59.48
Net reproductive rate, R_0	$\Sigma l_x m_x$	16.21
Mean generation time, T	$\Sigma l_x m_x(X)/\Sigma l_x m_x$	26.69
Intrinsic rate of increase, r	$\log R_0/T$	0.10
Finite rate of increase, lambda	e^r	1.11
Doubling time, DT	$\ln 2/r$	6.3

X, age of female parents at beginning of each interval.
m_x, expected number of daughters that will be produced by a female still alive at age X (maternity).
l_x, proportion of females that have survived to age X ('age specific survivorship', $l_0 = 1.00$).
R_0, number of daughters that replace an average female in 1 generation.
T, mean of the period during which daughters are produced.
r, number of new females per current female per day.

20 ± 2.8 days (range $= 4$–40 days), respectively. Fourteen percent of the males in the cohort had died just 3 days after emergence but none of the females. Within 3 weeks after emergence, only 34% of the males remained alive, compared with 50% of the females. Very few males (9%) survived beyond 35 days.

Six life table parameters were recorded from l_x and m_x values for *B. arisanus* females (Table 2). The net reproductive rate, R_0, suggested that 16 daughters will replace a female parent in 1 generation; thus, based on the intrinsic rate of increase, r, a 10% increase in the population is predicted each day; and, that *B. arisanus* could double its population in ≈ 6 days.

Discussion

The overlap in the emergence of *B. arisanus* and fruit flies (from host eggs that escaped parasitization) necessitates the development of a procedure that could segregate them to ensure clean cultures of parasitoids. We used a holding container (4.4 × 8.9-cm diam) fitted with a screen cover that had a mesh size (1 mm^2) wide enough to facilitate exit of parasitoids but not fruit flies. Notwithstanding, smaller flies managed to pass through the screen and contaminate our cultures occasionally. Normal-sized flies, on the other hand, succumbed as they forced their way into the screen mesh, partially sealing off exit holes for parasitoids. This technique may not be flawless but could be useful in small scale rearing of *B. arisanus*. Nevertheless, from the standpoint of mass production, segregation of parasitoids from fruit flies, preferably before adult eclosion, would be ideal in order to eliminate problems associated with sorting large number of parasitoids.

We determined that providing a female parasitoid with >20 fruit fly eggs for oviposition did not necessarily result in a dramatic increase in the mean number of parasitized eggs. Either the increment of parasitization was marginal or the plateau (at host clutch size of 100 eggs) was accompanied by a decrease in the level of host parasitization. The plateau may have indicated that oviposition by the female parasitoids had passed its optimum level. This behavior is typical of a Type I functional response where the interval between the time a female parasitoid first lays eggs and a search is again resumed becomes limiting (Holling, 1959). Apparently, it is not economically advantageous to provide *B. arisanus* with more hosts than necessary (Lawrence et al., 1978). Moreover, this finding is in concurrence with an earlier observation that a host clutch size to female parasitoid ratio of 20:1 is sufficient to optimize yield of parasitoids (Harris & Bautista, 1996) and compensate for the egg killing effect caused by *B. arisanus* oviposition (Newell & Rathburn, 1951).

We expected a higher incidence of superparasitized hosts when a clutch size of 25 fruit fly eggs was exposed to 5 parasitoid females for 24 h. Not only were very few hosts available for oviposition but also the exposure time to parasitoids was longer. Moreover, despite using potentially fecund females (15–16 days old post eclosion), <1 out of 100 eggs dissected (total of 4 replications) contained more than 1 parasitoid egg (range $= 0$–0.1). This finding may have indicated that

female *B. arisanus* was able to discriminate unparasitized from previously parasitized hosts (Ramadan et al., 1992; Lawrence et al., 1978). Thus, superparasitism, as a host mortality factor, may not be critical nor a constraint in the production of *B. arisanus*. Although superparasitism is more prevalent in the field where fruit fly eggs are patchy in distribution (Kaya & Nishida, 1968), this phenomenon is less common in the laboratory because gravid female parasitoids are provided with an ample supply of host eggs.

Initial productivity by *B. arisanus* was already apparent as early as maternal ages 1–5 days post eclosion. Considering that newly emerged females had a ready complement of matured eggs for oviposition (Ramadan et al., 1992), it is not surprising for some females to parasitize the hosts within 24 h after emergence. On the other hand, female productivity, which diminished considerably 3 weeks after emergence, was 2–3 times higher than those reported for wild *B. arisanus* (Ramadan et al., 1994) when comparison was based on mean live progeny produced per female per day. The discrepancy in fertility of female *B. arisanus* observed between these tests could be attributed to different sources of parasitoids assayed. We used parasitoids that had been colonized in the laboratory for some 150 generations thus, laboratory-adapted while those used by Ramadan et al. (1994) were bred in captivity for only three generations. Thus, the rearing pressures exerted by the 'new' environment (laboratory conditions) on the latter parasitoids may have resulted in lower fertility of reproducing females (Kajita, 1973; Raulston, 1975). Nevertheless, we likewise observed that the progeny sex ratio of laboratory-colonized *B. arisanus* was 40–70% females >2 weeks after female emergence indicating fertilization of parasitoid eggs as a result of successful mating between sexes. Our finding that female *B. arisanus* became less productive 3 weeks after emergence concurred with that reported by Ramadan et al. (1992; 1994) and should provide a basis to discard females older than 20 days for egging. Moreover, a dramatic shift in the progeny sex ratio toward males indicated that egging of female parasitoids should be done during early reproductive period to increase the likelihood of obtaining a sizeable complement of females in the progeny.

Considering that no demographic information is available on *B. arisanus*, the parameters we presented may be useful in facilitating efficient insectary propagation of this parasitoid. Nevertheless, further adjustments or refinements in the rearing procedures of the parasitoid should improve the productivity of the reproducing broods.

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