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Fecundity and oviposition of *Eucelatoria bryani*, a gregarious parasitoid of *Helicoverpa zea* and *Heliothis virescens*

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Abstract

We examined longevity, fecundity, and oviposition strategies of *Eucelatoria bryani* Sabrosky (Diptera: Tachinidae), a gregarious endoparasitoid of *Helicoverpa zea* (Boddie) and *Heliothis virescens* (F.) (Lepidoptera: Noctuidae). Longevity of adult female *E. bryani* was not related to body size. In contrast to longevity, larger *E. bryani* females had greater potential fecundity than smaller females, as determined by the number of embryonated eggs present in the common oviduct. However, female parasitoid size did not affect primary clutch size (number of eggs deposited in a host). Because embryos in eggs located in the ovisac were larger than those located elsewhere in the common oviduct, maximum primary clutch size may be physiologically limited by the number of fully mature eggs a female has available at one time. *E. bryani* females adjusted primary clutch size in response to host size, for both *H. zea* and *H. virescens*. This adjustment appears to be adaptive because females did not overexploit hosts by depositing more larvae than a host could support. Adult emergence was not related to host size. Although host weight positively influenced *E. bryani* progeny weight, increases in progeny size with host size were counterbalanced by increases in primary clutch size with host size.

Introduction

Intraspecific size variation among adult parasitoids can be large as a result of differing environmental factors they experience as larvae. Among factors that affect adult size are host quality and the amount of host resource available to each parasitoid in a particular host (Rabinovich, 1971; Tagaki, 1985). Because parasitoid size may be correlated with individual fitness, an understanding of reproductive attributes and how individual parasitoids interact with their hosts is essential to evaluating the impact of a parasitoid species on its host (Doutt *et al.*, 1976). Practically, the effectiveness of a parasitoid species as a biological control agent is linked to the quality of individual parasitoids (Ridgway & Morrison, 1985).

In the present study, we examine oviposition patterns and associated factors that influence progeny allocation by individual females of the gregarious larval endoparasitoid *Eucelatoria bryani* Sabrosky (Diptera: Tachinidae) and the fitness of those progeny. *E. bryani* has been considered a promising candidate for mass propagation and release for controlling *Helicoverpa zea* (Boddie) and *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) (Knipling, 1992). Because the size of laboratory-reared *E. bryani* can be highly variable (Ziser *et al.*, 1977; Mani & Nagarkatti, 1983; Reitz, unpubl.), we address the following specific questions: (1) Is adult female longevity related to body size? (2) Is potential fecundity of *E. bryani* related to female size, host species or host size? (3) Do females adjust clutch size in response to host size? (4) Is there a difference between primary clutch size (number of progeny oviposited in a host) and secondary clutch size (number of adults that develop from a host)? (5) Does host size or clutch size affect offspring size?

Materials and methods

Our colony of *E. bryani* was derived from colonies originally established from material collected in Arizona and maintained at USDA laboratories in College Station and Weslaco, TX. Adult flies were reared in groups of 100–200 in Plexiglas^{TM} cages (40 × 40 × 40 cm) and given a diet of sugar (sucrose) cubes and water. We used larvae of *H. zea* and *H. virescens* to rear *E. bryani*. *H. zea* and *H. virescens* larvae were derived from colonies maintained at USDA laboratories in Tifton, GA, and Stoneville, MS. We reared *H. zea* and *H. virescens* larvae individually in 31 ml plastic cups according to the methods described in Adler & Adler (1988). *E. bryani*, *H. zea* and *H. virescens* were maintained in an environmental chamber at 26 ± 2 °C, 70 ± 5% r.h. and a L14:D10 photoperiod. Voucher specimens are deposited in the Clemson University Arthropod Collection.

We used the length of the metathoracic tibia as a measure of body size for flies, as it is highly correlated with puparial weight (females, $r = 0.854$, $P < 0.001$, $n = 27$; males, $r = 0.815$, $P < 0.001$, $n = 28$), but does not fluctuate as does weight (Bai, 1986). Tibial length was measured by use of a stereomicroscope fitted with an ocular micrometer. Because clutch size values were small whole numbers, for all experiments involving clutch size, we transformed clutch size values by taking the square root of the sum of clutch size + 0.375 [i.e., $\sqrt{(Y + 0.375)}$, Sokal & Rohlf, 1981]. All means are reported as untransformed means ± SE. All statistical analyses were performed with the Statistical Analysis System (SAS Institute, 1989).

We determined adult longevity by rearing *E. bryani* as described above and checking daily for dead flies. After retrieving dead individuals, we measured their metathoracic tibial length and recorded their sex. We determined potential fecundity of *E. bryani* reared from both *H. zea* and *H. virescens* by dissecting, in Pringle's saline, healthy 2- to 2.5-week old flies that had not parasitized any hosts, and counting the number of embryonated eggs present in the common oviduct. To determine if potential fecundity increased with female size or differed for females reared from *H. virescens* or *H. zea*, we used an analysis of covariance (ANCOVA) on the number of eggs in the common oviduct, with host species as the treatment effect and *E. bryani* tibial length serving as a covariate.

To determine if embryonic development differed according to position in the oviduct, we measured the length and width of embryos in five eggs present in each of three different sections of the oviduct for eight females, reared from *H. zea*. The three sections were: (1) the ovisac, the enlarged, highly tracheated distal section of the common oviduct (Wood, 1987); (2) the section immediately anterior to the ovisac; and (3) the section at the anterior end of the common oviduct (i.e., where the two lateral oviducts unite).

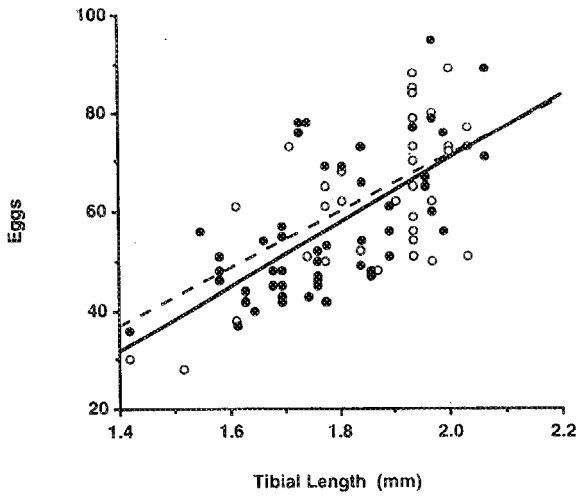

Fig. 1. The relationship between *E. bryani* size and potential fecundity, measured as the number of eggs present in the common oviduct. Potential fecundity increases significantly with size for both females reared from *H. zea* ($Y = -58.8 + 64.8X$, $P < 0.0001$; closed circles, solid line) and *H. virescens* ($Y = -43.0 + 57.4X$, $P < 0.0001$; open circles, dashed line). There is no significant difference in potential fecundity between females reared from *H. zea* and *H. virescens*.

For all tests involving parasitism, we used inexperienced 2- to 3- week old female flies, which had been isolated for approximately 2 h, to parasitize feeding stage larvae of either *H. zea* or *H. virescens*, as indicated. We weighed all larvae within 1 h of parasitization. To parasitize a larva, we gripped it with soft forceps behind the head capsule and presented it to an individual female fly in a Plexiglas^{TM} cage (30 × 30 × 40 cm) for a maximum of 2 min or until the fly oviposited in it, whichever came first. Oviposition can be detected by a drop of hemolymph appearing on the host cuticle after it has been pierced by the sternotheca of the female fly. Each fly was used only once. We cleaned the cage and forceps with 95% ethanol between each oviposition trial.

To determine if primary clutch size of *E. bryani* is greater than secondary clutch size, over a range of host sizes, we used third through fifth instars of *H. virescens*. Following parasitization, we sorted larvae into three size classes and randomly assigned half of the larvae

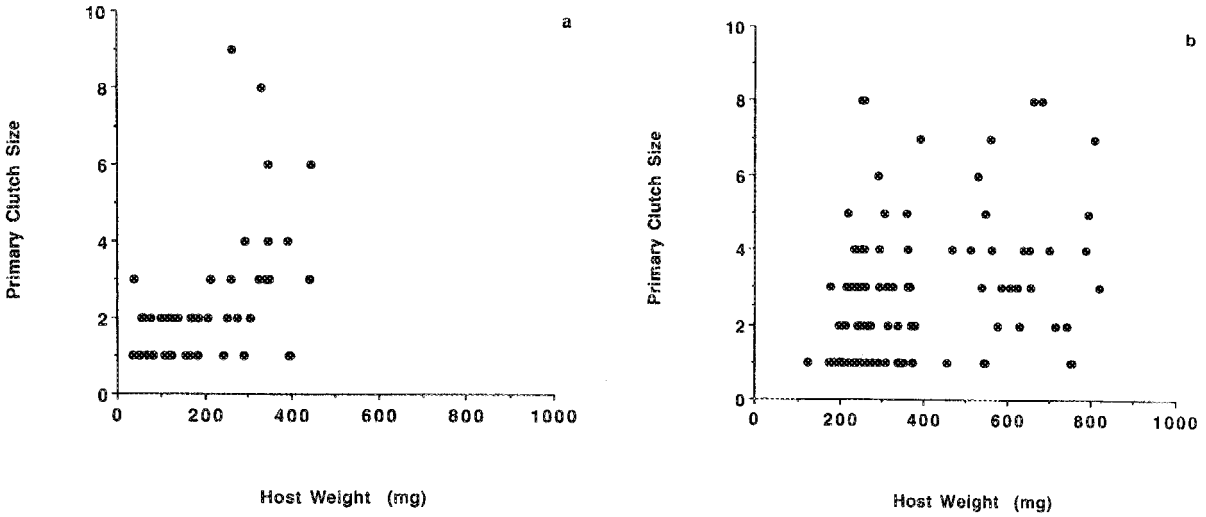

Fig. 2. (a) Primary clutch size of *E. bryani* when ovipositing in third through fifth instars of *H. virescens* (Y = 1.16 + 0.0021X, P<0.0001, for transformed data); (b) Primary clutch size of *E. bryani* when ovipositing in fifth instars of *H. zea* (Y = 1.47 + 0.001X, *P*<0.005, for transformed data).

in each size class to one of two treatment groups. We dissected the larvae in one group (n = 53) immediately following parasitization and counted the number of parasitoid maggots present (primary clutch size). We held the remaining group of larvae (n = 53) individually until *E. bryani* maggots had pupariated. Then we transferred the puparia to individual containers and recorded the number of adult flies that successfully emerged from each host (secondary clutch size).

To determine if primary clutch size was greater than secondary clutch size in *H. virescens*, we used an analysis of covariance (ANCOVA) on the transformed clutch size values, with primary and secondary clutches as the treatment effect and host weight serving as the covariate.

We also compared primary and secondary clutch sizes when *E. bryani* parasitized *H. zea*. To determine if primary clutch size was greater than secondary clutch size, we parasitized fifth instars of *H. zea* (n = 97). After parasitization, we returned larvae to individual containers. After the *E. bryani* maggots had pupariated, we placed them individually in 31-ml cups until adult emergence, and then we examined the host carcasses for any maggots that failed to pupariate. We then compared clutch size differences to determine if primary clutch size was greater than secondary clutch size.

To determine if primary clutch size varies according to female size, we used a subset of the parasitized fifth instars of *H. zea* (n = 63). This is the most preferred host stage for *E. bryani* (Martin *et al.*, 1989), and we expected that each fly would be more likely to oviposit its maximum clutch in these larger larvae. Following parasitism, we dissected hosts and counted the number of maggots deposited in each host (primary clutch size) and measured the length of each female fly's metathoracic tibia. Then we regressed the transformed clutch size values on female tibial length and host weight.

To determine if the size of *E. bryani* progeny is related to host size and primary clutch size, we used progeny reared from *H. zea* (n = 97), as described above. We weighed *E. bryani* puparia 9 days following parasitization, recorded their sex upon adult emergence and dissected any puparia that failed to eclose to determine sex, where possible. To determine if host weight and primary clutch size affect the size of *E. bryani* progeny, we regressed the weighted mean of male and female puparial weights on values for host weight and primary clutch size. For this analysis, we included only puparia that produced adult flies.

Results

Female size, longevity and potential fecundity. Female longevity (17.2 ± 1.43 days) was not related to tibial length ($r = -0.162$, P>0.32, n = 39). Common oviducts of larger females contained significantly more

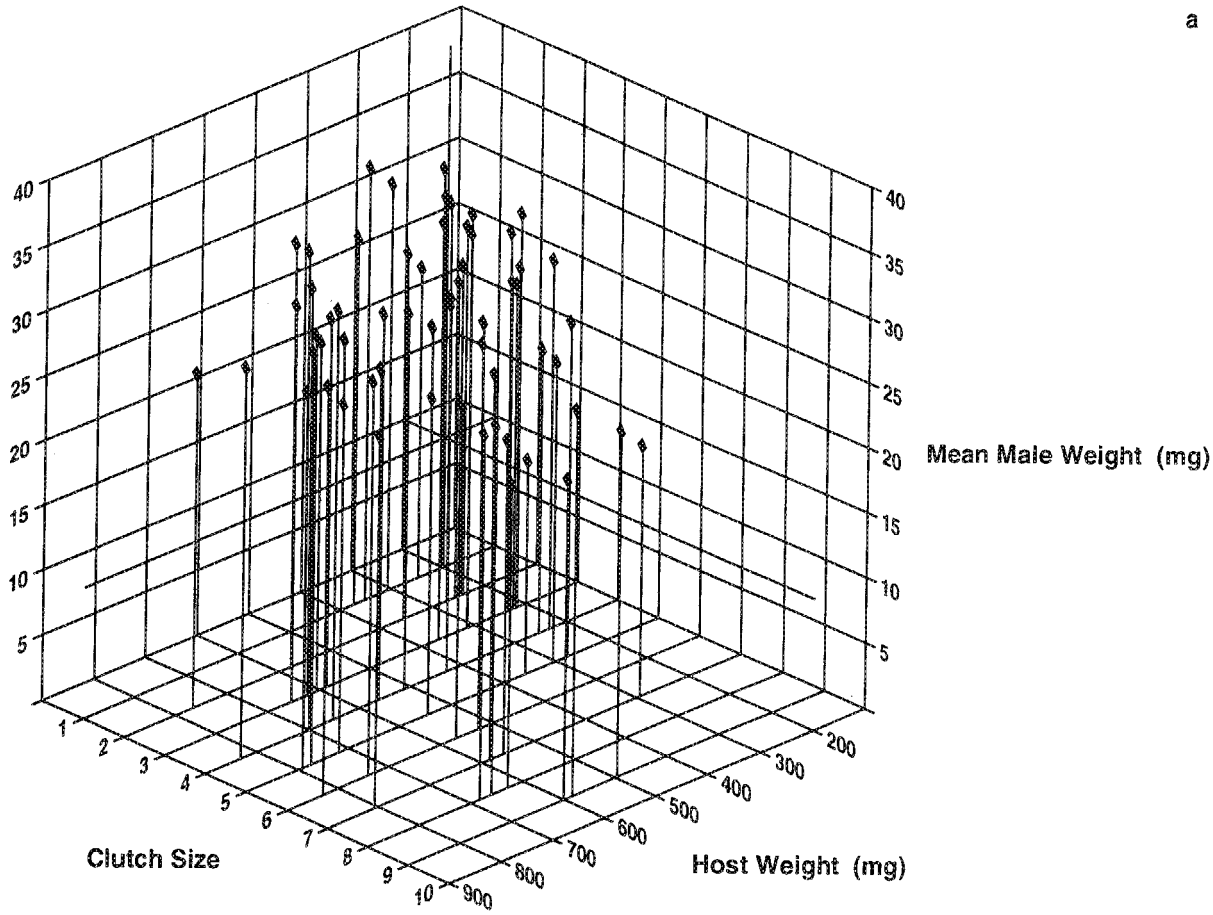

Fig. 3. Relationship between mean puparial weight per clutch for *E. bryani*, *H. zea* host weight and primary clutch size. (a) male progeny: Y1 = 28.46 + 0.01X1 − 2.76X2 where Y1 = (mean puparial weight of males in a clutch, in mg), X1 = (host weight, in mg), X2 = (primary clutch size, transformed values); (b) female progeny: Y2 = 29.81 + 0.01X1 − 3.43X2 where Y2 = (mean puparial weight of females in a clutch, in mg), X1 = (host weight, in mg), X2 = (primary clutch size, transformed values).

eggs than did the oviducts of smaller females ($F = 65.6$, $P<0.0001$, df = 1, 98, Fig. 1), but there was no significant difference in potential fecundity between females reared from *H. zea* or *H. virescens* ($F = 0.87$, $P<0.35$, df = 1, 98). The number of eggs present in the oviduct ranged from 36 to 95 (56.12 ± 1.99) for females reared from *H. zea*, and 28 to 89 (62.98 ± 2.10) for females reared from *H. virescens*. Tibial length ranged from 1.42 mm to 2.07 mm (1.78 ± 0.020 mm) for females reared from *H. zea*, and 1.42 mm to 2.07 mm (1.85 ± 0.023 mm) for females reared from *H. virescens*.

Embryos (still enclosed in their egg chorion) in the oviduct also showed variation in size and development. Those located in the expanded ovisac (Section 1) were significantly larger than embryos elsewhere in the oviduct (Table 1). Embryos located closest to the lateral oviducts (Section 3) were significantly smaller than those in the other two sections (Table 1). Also, based on their body color and degree of sclerotization, the embryos in Section 3 appeared less developed than those in the other two sections of the oviduct.

Female size and primary clutch size. When ovipositing in fifth instars of *H. zea*, primary clutch size of *E. bryani* was not influenced by female size ($F = 0.12$, df = 1, 60, $P>0.73$). However for this subset of data, primary clutch size did increase with increasing weight of *H. zea* hosts ($F = 10.0$, df = 1, 60, $P<0.01$). The mean primary clutch size deposited in these hosts was 2.86 ± 0.23 range 1–8), and the mean female tibial length was 1.81 ± 0.02 mm (range 1.23–2.10 mm). Because female size was not significant, we did not

b

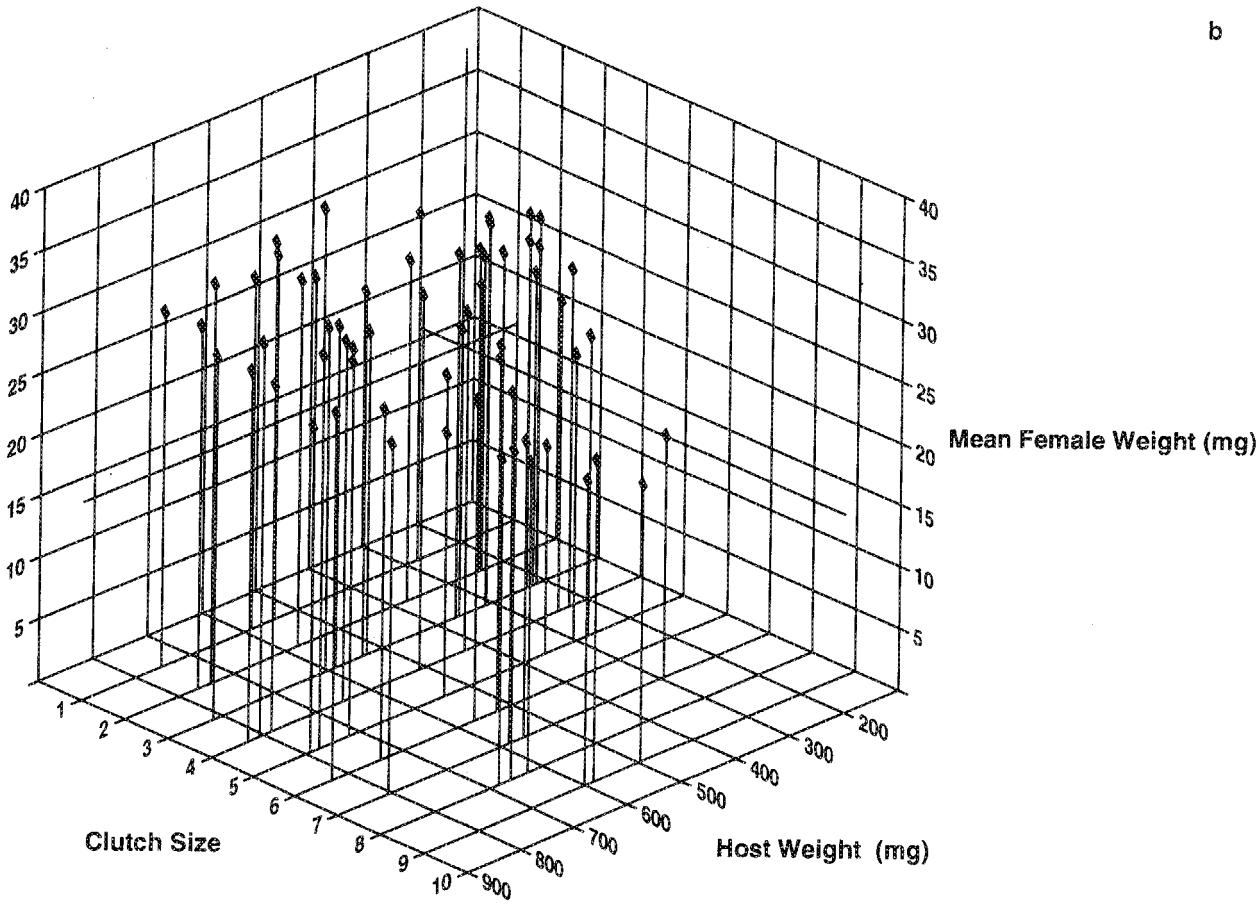

Fig. 3. Continued.

Table 1. Dimensions of *Eucelatoria bryani* embryos in eggs in three sections of the common oviduct. Section 1 is the ovisac, Section 2 is the common oviduct immediately anterior to the ovisac, Section 3 is the anterior section of the common oviduct adjacent to the lateral oviducts. Means ± SE are based on 5 embryos per female ($n = 8$)

Section	Length (mm)*	Width (mm)*	Length × Width (mm^2)*
1) Ovisac	0.725 ± 0.0116^a	0.390 ± 0.0032^a	0.224 ± 0.00442^a
2) Middle	0.552 ± 0.0123^b	0.251 ± 0.0062^b	0.141 ± 0.00617^b
3) Anterior	0.433 ± 0.0053^c	0.167 ± 0.0023^c	0.073 ± 0.00153^c

* Means within a column followed by the same letter are not significantly different ($P > 0.01$, Tukey multiple comparison method)

include it in further clutch size analyses.

Primary and secondary clutch size. Primary clutch size (2.28 ± 0.22) was not significantly greater than secondary clutch size (2.51 ± 0.26) when *E. bryani* females oviposited in third through fifth instars of *H. virescens* ($F = 0.15$, df = 1, 102, $P > 0.70$). However, females did oviposit more progeny in larger

hosts ($F=39.5$, df = 1, 102, $P<0.0001$, Fig. 2a). When *E. bryani* oviposited in fifth instars of *H. zea*, primary clutch size increased with increasing weight of the *H. zea* hosts ($F=8.41$, df = 1, 95, $P<0.005$, Fig. 2b). The mean primary clutch size was 2.90 ± 0.19. Primary clutch size was greater than secondary clutch size (mean difference 0.27 ± 0.05, $t=4.4$, df = 95, $P<0.0001$, paired t-test). However, there was no larval mortality and 94% of *E. bryani* progeny (n = 281) developed to adults. Only 18 individuals (6%) failed to complete pupation and emerge as adults. Differences in primary and secondary clutch size were not related to host weight ($F=0.90$, df = 1, 95, $P>0.35$). Therefore, differences among parasitized hosts in the number of emerging flies resulted from variation in the number of eggs deposited in the host and not from larval mortality within the host.

Host size, clutch size and progeny size. In examining the effects of host weight and primary clutch size on the weight of *E. bryani* puparia, we found host weight and primary clutch size had similar effects on the size of males and females (Fig. 3a, b). For both male and female *E. bryani*, puparial weight increased with the weight of the *H. zea* host (t-test for slopes: males, $t=4.11$, df = 59, $P<0.0001$; females, $t=5.58$, df = 59, $P<0.0001$). Increasing primary clutch size constrained increases in the puparial weights of male *E. bryani* ($t=-2.80$, df = 59, $P=0.007$), and the puparial weights of females ($t=-5.24$, df = 59, $P<0.0001$).

Discussion

Potential fecundity of *E. bryani* was positively related to female size for flies reared from both *H. zea* and *H. virescens*. This result is in agreement with that of Mani & Nagarkatti (1983) who found that larger females of *E. bryani*, reared from *Helicoverpa armigera* (Hübner), tended to have more eggs present in the oviduct than smaller individuals, and is typical of other tachinids (King *et al.*, 1976), as well as some hymenopteran parasitoids (Charnov *et al.*, 1981; Tagaki, 1985, Bai *et al.*, 1992, Hohmann *et al.*, 1988, Croft & Copland, 1993; Pavlik, 1993). Because there was no difference in potential fecundity between flies reared from *H. zea* and *H. virescens*, these two species are equally suitable for the development of *E. bryani*. In contrast to potential fecundity, female longevity was not affected by female size. Our results show potential fecundity is similar to the mean numbers of puparia produced over the lifetime of a female, as found by Bryan *et al.* (1972), who did not report female size, and are in agreement with estimates of fecundity for tachinids with life histories similar to that of *E. bryani* (Dowden, 1933; Townsend, 1940). Although tachinids with similar embryonic development to that of *E. bryani* continue to produce eggs throughout their lifespan (Kugler & Nitzan, 1977), the rate of production probably decreases over time, as the ovaries degenerate with age. Therefore, the number of eggs present in the oviduct at any one time is a useful, albeit conservative, estimate of the potential lifetime fecundity for *E. bryani*. Given these findings, female size, which reflects the number of eggs present in the oviduct, can be used as a measure of potential lifetime reproductive output of *E. bryani*.

Our results indicate that *E. bryani* can successfully parasitize a wide range of host sizes, and females adjust oviposition in manner so as not to overexploit or underexploit host resources. Martin *et al.* (1989) found that *E. bryani* emergence and puparial weights were generally positively related to host developmental stage. However, their assessment was based on superparasitized hosts. Females compensate for differences in host sizes by adjusting clutch size according to host size. In these singly parasitized hosts there was little parasitoid mortality. Differences between primary and secondary clutch sizes in the two host species result from how those data were measured and probably not from differences in host quality. In addition, there was little variation in progeny size from hosts of different sizes.

Considering that hosts continue to grow during the initial stages of parasitoid development (Brewer & King, 1980), additional factors, possibly tactile or visual cues of the condition of the host, probably play a role in determining primary clutch size that *E. bryani* deposits. Oviposition behavior of *E. bryani* is variable; females can make rapid oviposition attacks lasting less than 1 s, or they may stay in contact with the host for several seconds before ovipositing (Reitz, unpubl.). Although female size did not significantly affect primary clutch size, an additional factor in progeny allocation by *E. bryani* may be the number of mature eggs a female has available at one time. Embryos located in the ovisac were larger and appeared more developmentally advanced than those located elsewhere in the oviduct. In addition, the largest primary clutch size we recorded in this study was 9, which coincides with the greatest number of eggs we found in the ovisac of

dissected females. Nevertheless, the negligible larval mortality and little variation in mean puparial weights per clutch suggest *E. bryani* females adjust clutch size in an adaptive manner that does not overexploit or underexploit host resources.

Given that the size of laboratory-reared flies can be manipulated by the number of maggots per host (Ziser *et al.*, 1977) or by the amount of artificial media available per maggot (Bratti & Nettles, 1992), understanding individual variation in fecundity and progeny allocation will assist in determining the most efficient method for rearing *E. bryani* for inundative releases. In addition, these results indicating that *E. bryani* can exploit a wide range of *H. zea* and *H. virescens* stages and sizes and is not dependent on a particular host size or stage will assist in assessing the impact of *E. bryani* on populations of *H. zea* and *H. virescens* (Knipling, 1992).

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