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Parasitism of the grasshopper *Melanoplus sanguinipes* by a sarcophagid fly, *Blaesoxipha atlanis*: influence of solitary and gregarious development on host and parasitoid

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Abstract

Blaesoxipha atlanis (Aldrich) (Diptera: Sarcophagidae) is a common parasitoid of the grasshopper *Melanoplus sanguinipes* (F.) (Orthoptera: Acrididae) in western Canada. We tested the hypothesis that *B. atlanis* can develop as either a solitary or a gregarious parasitoid, and assessed the influence of parasitism on the growth and survival of infected grasshoppers. Males and females of *M. sanguinipes* were parasitized manually with one, two, or three first-instar larvae of *B. atlanis* in the laboratory. Parasitism was more deleterious to males than females of *M. sanguinipes*; females are larger than males. Host survival and longevity declined with the number of larvae per host in a sex-specific manner. In females, 39%, 24%, and 8% of hosts containing, respectively, one, two, and three sarcophagid larvae survived parasitism. Although 41% of single-parasitized males survived, all males containing more than one larva died. Variations in host quality as measured by dry mass explained much of the response to parasitism in male, but not female, hosts. Parasitoid larvae, apparently, did not cause significant physical damage to host organs and tissues but instead functioned as a metabolic sink. The greater metabolic activity associated with egg production could account for the relatively higher tolerance to parasitism of female, as opposed to male, grasshoppers. Developmental time, adult size, and percentage survival of *B. atlanis* declined with the intensity of parasitism, especially in parasitoids developing in male hosts. Females developing gregariously contained fewer ovarioles at eclosion than counterparts developing as solitary larvae. The mean body size of field-collected *B. atlanis* did not differ from that of laboratory-reared parasitoids developing singly in a host. Gregarious development is an alternative strategy to solitary development that may enable *B. atlanis* to maintain population numbers during periods of grasshopper scarcity.

Introduction

Most species of Sarcophagidae feed as larvae on decaying animal and plant matter (Rees, 1973), but a small number are parasitic on vertebrates and arthropods (Danks, 1979; Shewell, 1987). Sarcophagid flies are some of the most important parasitoids of grasshoppers in North America (Rees, 1973). For example, *Blaesoxipha atlanis* (Aldrich) is a known parasitoid of some 14 species of acridids, including *Melanoplus sanguinipes* (F.), on the grasslands of southern Alberta, Canada. A pest of cereal crops and rangeland (Johnson, 1989), *M. sanguinipes* is often found in weedy, uncultivated areas adjacent to cereal plantings. Parasitoids emerge from overwintering sites in June and are potentially multivoltine. In samples collected in southern Alberta between 1993 and 1997, the percentage of adult *M. sanguinipes* that were parasitized by sarcophagids ranged from 2% to 13%, with *B. atlanis* being the most common among five species

of flies (T. Danyk & D. L. Johnson, unpubl.). From the majority of grasshoppers a single larva of *B. atlanis* emerged; however, more than one larva emerged from some hosts, which suggests gregarious development. The presence of more than one larva in hosts may result from multiple larvipositions by the same female, single larvipositions by different females, or a combination of both.

Gregarious development has been studied extensively in the parasitic Hymenoptera (reviewed in Godfray, 1994). In solitary species, supernumerary larvae are normally eliminated in the first instar by physical combat and/or physiological suppression (Mackauer, 1990). In contrast, larvae of gregarious species do not fight for host possession, and supernumerary larvae are eliminated generally by exploitative competition for host resources. In the parasitic Diptera, the presence of more than one larva in a given host occurs frequently in the Conopidae, Phoridae, Tachinidae (Feener & Brown, 1997) and in some Cecidomyiidae (Mackauer & Foottit, 1979). Although little is known about gregarious development in sarcophagid parasitoids, research on non-parasitic species has shown that resources are utilized during larval development, apparently, by exploitative competition in a manner similar to that observed in gregarious parasitoid wasps. For example, increased densities of larvae in rearing media resulted in shorter developmental time and lower mean larval dry mass in *Sarcophaga bullata* Parker (Baxter & Morrison, 1983), reduced dry mass of puparia in *Pecia abnormalis* Enderlein (Tanaka et al., 1990), and lower survivorship, developmental time, dry mass, and fecundity in *Boettcherisca formosensis* Kiner & Lopes (So & Dudgeon, 1989).

We tested the hypothesis that *B. atlanis* can develop as either a solitary or a gregarious parasitoid. The survival of more than one larva should vary with host quality, i.e., the total amount of nutritional resources available (Mackauer & Sequeira, 1993). If parasitoid growth is constrained by insufficient resources due to small host size, the surviving parasitoids should be smaller, develop for longer, or both. To test the influence of host size on parasitoid growth and fecundity, we used males and females of *M. sanguinipes* as hosts; males are smaller than females (Vickery & Kevan, 1983). In addition, we assessed the influence of solitary and gregarious parasitism on the growth and survival of infected grasshoppers. We discuss solitary and gregarious development as alternative reproductive tactics in *B. atlanis*.

Materials and methods

Insect colonies. Females of *B. atlanis* are larviparous and, in southern Alberta, start producing offspring in early July when grasshoppers are in the third or fourth instar. Females attack both active and resting grasshoppers, usually from the posterior end, and deposit one or more larvae. Attacked insects may use their hind legs to dislodge newly deposited larvae. The larvae cut a hole in the host's integument with their sickle-shaped mandibles and develop as endoparasitoids within the hemocoel, feeding on hemolymph and fat body (Rees, 1973). After 5–7 days at 25 °C, larvae of *B. atlanis* emerge as third-instar maggots through a hole made in the dorsal region of the membrane connecting head and thorax (T. Danyk & D. L. Johnson, unpubl.). The larvae enter the soil in the fall to overwinter and pupariate in the following spring.

A laboratory colony of *B. atlanis* was established from insects collected on land bordering cereal crops in southern Alberta. Parasitoids were reared on a non-diapausing strain of *M. sanguinipes* (Pickford & Randell, 1969), which was fed on wheat leaves (*Triticum aestivum* L. cv. 'Katepwa'), commercially grown head lettuce (*Lactuca sativa* L.), and wheat bran. Adult flies were maintained at 22° ± 2 °C, a L16:D8 light-dark regime, and fed with sucrose and deionised water *ad libitum*.

Estimating host quality. Body size as measured by dry mass (DM) at parasitization is used often as a proxy of host quality (Nicol & Mackauer, 1999), which latter can be defined as the total amount of nutritional resources available to the immature parasitoid for growth and development (Mackauer & Sequeira, 1993). Dry mass is less subject to variation than wet mass (WM), which varies with the insects' feeding schedule; however, DM can be determined only by destructive sampling. We estimated initial host size, therefore, by measuring WM and converting WM to DM. From a stock colony, 40 males and 40 females of *M. sanguinipes* were selected and weighed individually on a Mettler AE50 balance to the nearest milligram (WM_0). Next the insects were frozen for 24 h, dried in an oven at 55 °C for 48 h, and weighed on the same balance (DM_0). The regressions of DM_0 on WM_0 were sex-specific (ANCOVA, difference between slopes [$F_{1,76} = 4.58$; $P = 0.036$] and y-intercepts [$F_{1,76} = 6.72$; $P = 0.012$]):

$$DM_{0[male]} = 0.418WM_0 - 36.1, \qquad (1a)$$

$$DM_{0[female]} = 0.504WM_0 - 79.0, \quad (1b)$$

with the SE of the estimate = 7.7 ($r = 0.90$; $P < 0.001$) and 8.9 ($r = 0.98$; $P < 0.001$) for Equations (1a) and (1b), respectively.

Effect of single and multiple parasitism on grasshoppers and parasitoids. Preliminary observations indicated that the intensity of *B. atlanis* infections in the field ranged from one to three larvae per grasshopper, with one larva being the most and three larvae the least common. In Experiment 1, we tested if *B. atlanis* can facultatively develop as a gregarious parasitoid by infecting 6- to 12-day-old males and females of *M. sanguinipes* in four groups: three groups were infected with, respectively, one, two, or three larvae of *B. atlanis;* and the fourth group was unparasitized, serving as a control. The experiment was replicated once (replicate 1, $n = 150$; replicate 2, $n = 145$). Grasshoppers were weighed individually to obtain their WM_0. Next the right fore and hind wings of each grasshopper were surgically removed, and the right tympanum was ruptured mechanically with a needle. This procedure facilitated successful infection by the parasitoid. To obtain infective first-instar larvae of *B. atlanis*, we removed the ovisacs of 11- to 13-day-old females ($n = 36$) that had been lightly anaesthetised with CO_2. The ovisacs were placed on filter paper moistened with deionized water and ruptured, releasing the larvae. One or more larvae (from different females) were transferred individually with a camel's hair brush and placed directly on the ruptured tympanum of each host.

Grasshoppers were reared individually in 250 ml, clear plastic containers which were kept in a controlled environment chamber at 25 °C (day) and 20 °C (night) and a L16:D8 light-dark regime. They were provided with wheat leaves *ad libitum*, at the same time each day. For pupariation, emerged larvae of *B. atlanis* were placed on sand and returned to the environment chamber. Flies emerging from the puparia were left in the chamber for 1 day, then frozen for 24 h, dried at 55 °C for 24 h, and weighed individually on a Cahn 29 automatic electrobalance to the nearest microgram to determine their dry mass (DM_p). As a proxy of body length, the length of the right hind femur (HFL) of each fly was measured under a dissecting microscope equipped with an ocular micrometer at 40× magnification.

Under the given experimental conditions, larvae of *B. atlanis* emerged from grasshoppers within 9 days after infection. Grasshoppers from which not all the implanted larvae had emerged within that period were dissected. All grasshoppers were weighed to determine their dry mass at parasitoid eclosion (DM_e). Individual measurements of DM_e were corrected for the loss of the surgically removed wings by the addition of the mean mass of fore and hind wings estimated from a sample of each of 20 male and female wings. These corrected values of DM_e were compared with the DM_0 obtained by Equations (1a) and (1b). Puparia containing dead parasitoids, dead larvae remaining in the sand, and dead parasitoids dissected from infected grasshoppers were also dried in an oven and weighed.

Size and fecundity of parasitoids. Females of *B. atlanis* produce only one clutch of eggs and do not require an exogenous source of protein for egg maturation (T. Danyk, unpubl.). Therefore, the number of ovarioles at emergence determines a female's maximum lifetime fecundity or her potential fitness. In this paper, we shall confine our discussion to potential fitness only.

To assess the relationship between body size and fecundity in females of *B. atlanis*, we collected puparia from a laboratory colony (Experiment 2). The puparia were placed individually in gelatin capsules (size 000; T.U.B. Enterprises, North Augusta, Ontario) and stored at room temperature (22° ± 1 °C). At 24-h intervals, cohorts of eclosed flies were transferred from the capsules to separate 2 l plastic containers, provided with sucrose and water for 48 h, and stored at 4 °C. For dissection, the flies were lightly anaesthetized with CO_2. The ovisacs were removed, placed in a 0.8% aqueous solution of NaCl, and the total number of ovarioles was counted under a dissecting microscope.

Estimating intensity of parasitism by B. atlanis *in the field.* The results of Experiment 1 showed that the adult body size of *B. atlanis* varied with the number of larvae developing in each host. We used this information to estimate the frequency of solitary and gregarious development in the field by comparing the distributions of HFL between males and females of *B. atlanis* collected in the field and individuals developing as solitary and gregarious larvae in the laboratory. Adult parasitoids were collected with a sweep net along the margins of cereal fields, frozen, pinned, and stored in an insect cabinet. Only male Sarcophagidae can be reliably identified to species based on the genitalia as the final distinguishing characteristic (Aldrich, 1916). Additional samples of males were obtained from parasitized hosts collected in the field.

Data for females were obtained from individuals collected in the field and used to establish single-female laboratory colonies yielding male offspring for identification. Measurements of HFL from field-collected flies were compared with the mean HFL of male and female *B. atlanis* that eclosed from single-, double-, and triple-parasitized hosts in the laboratory.

Data analysis. Only grasshoppers from which all implanted larvae were recovered were included in the analysis. Events such as host death and parasitoid eclosion were assumed to have occurred at the midpoint between two successive feeding periods and were recorded as median times post-parasitization. We assessed the statistical significance of differences between treatment means by ANOVA and ANCOVA (with DM_0 as a covariate), using PROC ANOVA and PROC GLM, respectively, in the SAS package of computer programs (SAS Institute, 1988). Hypotheses were tested using the RANDOM function. Interaction terms in the model generated by ANCOVA were tested by pairwise comparisons of least-squares means by the LSMEANS function provided in GLM. Tukey's studentized range tests in GLM was used for multiple comparisons between means, with $\alpha = 0.05$ for significance. We used PROC REG to perform multiple linear forward-stepwise regression, with $\alpha = 0.05$ for the inclusion of a variable in the final model. For the analysis of host survival, the median times post-parasitization were transformed into values of 'lifeloss'. Lifeloss measures the survival of a parasitized grasshopper relative to that of unparasitized individuals. Because under the given experimental conditions all but one of the control insects survived to the age of 225 h post-parasitization, we estimated lifeloss by subtracting from 225 h the observed survival time of a parasitized grasshopper; this value was multiplied by -1 to obtain a positive integer. However, we did not determine the actual longevity of unparasitized grasshoppers, which was much longer than the 9-day duration of the experiment. *Z*-tests were utilized to discriminate between percentages (Johnson, 1980). Median values were determined using SYSTAT (Wilkinson, 1990), and the statistical significance ($\alpha = 0.05$) of differences between these values was determined by a Wilcoxon rank-sum test as provided in PROC NPAR1WAY of SAS. We used PROC NLIN to estimate the parameters of a non-linear regression model of parasitoid fecundity, and PROC RSREG to estimate the parameters of polynomial regression models describing the trade-off response functions between parasitoid mass and fecundity. The frequency distributions of HFL of field- and laboratory-reared flies were compared by a Kolmogorov-Smirnov two-sample test (Sokal & Rohlf, 1995, pp. 434–439).

The initial DM_0 of grasshoppers used as hosts in Experiment 1differed between replicates 1 and 2 (see below). We analysed all data, and the complete results are stated in the text; however, only the data of the first replicate are given in the tables to simplify presentation.

Results

Host quality. Host quality as measured by the initial DM_0 of males and females of *M. sanguinipes* did not differ between treatment groups (i.e., cohorts of hosts containing 0, 1, 2 or 3 parasitoids) within sexes (ANOVA, $F_{3,286} = 0.27$; $P = 0.85$). However, individuals in the second replicate were larger, on average, than those in the first replicate ($F_{1,286} = 44.07$; $P < 0.001$), and females were larger than males in both replicates (Tukey's test) (Table 1).

Survival of parasitized grasshoppers. Early death (= lifeloss) varied with the number of parasitoid larvae developing in each host (Table 2). Although the factors replicate and host sex alone were not significant, the interaction of replicate × treatment was significant because LSMEAN lifeloss of single-parasitized hosts in the first replicate was greater than that in the second replicate ($P < 0.001$). In addition, the interaction of treatment × host sex was significant because LSMEAN lifeloss of male hosts containing two ($P < 0.001$) and three parasitoids ($P < 0.001$) exceeded that of similarly parasitized female hosts (Table 1). The covariate DM_0 was also significant.

Because males and females of *M. sanguinipes* appeared to differ in their response to parasitism, we used multiple linear forward-stepwise regression to examine data separately by host sex; only data from hosts that died during the experiment (lifeloss > 0 h) were included. In male hosts, lifeloss was significantly correlated with treatment (T_p) ($r = 0.67$; $F_{1,92} = 73.73$; $P < 0.001$) and DM_0 ($r = -0.34$; $F_{1,91} = 24.03$; $P < 0.001$). The final model explained 56.1% of the variation in lifeloss ($F_{2,91} = 58.11$; $P < 0.001$):

$$\text{Lifeloss}_{[\text{male}]} = 23.835T_p - 0.523DM_0 + 119.596. \quad (2a)$$

Table 1. Changes in body mass and survival of males and females of *Melanoplus sanguinipes* in unparasitized individuals and individuals parasitized with one, two, or three larvae of *Blaesoxipha atlanis.* (Only data from the first of two replicates are shown; results from the second replicate were similar. See text for complete statistical analysis)

Host sex	Larvae per host	*n*	DM_0[a] (mg) mean (SE)	Change in DM[b] (mg) mean (SE)	Lifeloss[c] (h) mean (SE)	Mortality (%)
Male	0	21	114.1 (3.1)	−1.9 (1.4)	0	0
	1	22	111.3 (3.0)	−25.1 (1.7)	63.3 (9.9)	72.7
	2	20	114.0 (4.0)	−30.1 (1.1)	114.0 (4.2)	100.0
	3	14	108.9 (4.8)	−31.2 (2.0)	133.7 (4.9)	100.0
Female	0	21	157.4 (11.0)	+14.9 (6.4)	0	0
	1	20	170.4 (8.3)	−29.6 (3.1)	61.2 (9.9)	75.0
	2	18	146.6 (8.4)	−36.0 (4.0)	70.7 (10.5)	77.8
	3	14	142.8 (11.9)	−33.5 (4.4)	102.9 (8.9)	92.9

[a]DM_0 = initial dry mass at parasitization.
[b]Positive and negative signs indicate that hosts, respectively, gained or lost dry mass relative to DM_0 during parasitism.
[c]Reduction in longevity experienced by parasitized insects in comparison to unparasitized controls (larvae = 0).

Table 2. Analysis of covariance of 'lifeloss'[a] in males and females of *Melanoplus sanguinipes* parasitized with zero, one, two or three larvae of *Blaesoxipha atlanis*

Variable	df	F	P
Replicate	1, 2.77	1.54	0.309
Treatment [b]	3, 5.12	16.07	0.005
Host sex	1, 3.02	1.71	0.282
Replicate × treatment	3, 3.04	9.14	0.050
Treatment × host sex	3, 2.93	16.61	0.024
Replicate × host sex	1, 3.44	0.41	0.561
Replicate × treatment × host sex	3, 278	0.47	0.707
DM_0[c]	1, 278	7.11	0.008

[a]Reduction in longevity (h) experienced by parasitized insects in comparison to unparasitized controls (larvae = 0).
[b]Hosts containing zero, one, two or three parasitoid larvae.
[c]Dry mass (mg) of hosts at parasitization.

In female hosts, the regression model explained 22.6% of the variation in lifeloss ($F_{2,72} = 10.50$; $P < 0.001$), with T_p ($r = 0.41$; $F_{1,73} = 14.65$; $P < 0.001$) and DM_0 ($r = 0.24$; $F_{1,72} = 5.45$; $P = 0.022$) being significant:

$$\text{Lifeloss}_{[\text{female}]} = 13.782T_p - 0.165DM_0 + 91.770. \quad (2b)$$

The percentage of hosts surviving parasitism differed between male and female grasshoppers within treatment groups, with the exception of single-parasitized insects (Table 1). Whereas all double- and triple-parasitized male hosts died, 24.3% and 8.0% of the similarly parasitized female hosts survived (*z*-tests).

Host growth. Unparasitized *M. sanguinipes* continued to grow during early adult life, with females gaining relatively more in DM than their male counterparts (Tukey's test) (Table 1). In parasitized grasshoppers, however, the final DM_e was less than the DM_0 at the beginning of parasitism (Tukey's test). The amount of DM lost due to parasitism did not differ (Tukey's test) between males and females containing the same number of larvae despite the fact that female hosts were on average 33% larger in terms of DM_0 than males.

The DM_e of *M. sanguinipes* at the end of parasitism was correlated with DM_0 and could be predicted from it. For unparasitized hosts, the regressions of DM_e on DM_0 were sex-specific (male, $r = 0.90$; $F_{1,39} = 174.01$; $P < 0.001$; female, $r = 0.80$; $F_{1,40} = 72.98$; $P < 0.001$):

$$DM_{e[\text{male}]} = 0.678DM_0 + 35.448; \quad (3a)$$

$$DM_{e[\text{female}]} = 0.442DM_0 + 107.211. \quad (3b)$$

When adjusted for the same initial DM_0, females of *M. sanguinipes* gained DM faster than their male counterparts over the same period (ANCOVA, difference between slopes [$F_{1,79} = 4.12$; $P = 0.046$] and *y*-intercepts [$F_{1,79} = 31.61$; $P < 0.001$]).

Likewise, in parasitized hosts, the regressions of DM_e on DM_0 were sex-specific (ANCOVA, difference

between slopes [$F_{1,208} = 1.71$; $P = 0.19$] and *y*-intercepts [$F_{1,208} = 6.39$; $P = 0.012$]):

$$DM_{e\ [male]} = 0.732DM_0 + 1.143; \quad (4a)$$

$$DM_{e\ [female]} = 0.644DM_0 + 20.673. \quad (4b)$$

DM_e was correlated with DM_0 in male ($r = 0.88$; $F_{1,109} = 359.63$; $P < 0.001$) and female ($r = 0.88$; $F_{1,99} = 325.09$; $P < 0.001$) hosts. This indicates that hosts were unable to replenish DM assimilated by parasitoids during development and, furthermore, that parasitoids used a consistent proportion (i.e., the maximum amount available) of resources within hosts.

Parasitoid development. The percentage of infected *M. sanguinipes* in which all parasitoid larvae completed development declined with larval density (*z*-test) (Table 3). Similarly, the time from implantation to larval emergence decreased with an increase in the number of larvae per host (Wilcoxon test). Solitary larvae of *B. atlanis* developed at the same rate in both male and female hosts (Wilcoxon test), but gregariously developing larvae emerged earlier from male than female hosts (Wilcoxon test).

The mean total DM_p of parasitoids increased with the number of larvae per host (Tukey's test) (Table 3) although the average DM of individual parasitoids declined (Tukey's test). The total DM_p was greater for parasitoids developing as gregarious larvae in female, as opposed to male, hosts (Tukey's test), but host sex did not influence the DM_p of parasitoids that developed as solitary larvae (Tukey's test) (Table 3). Multiple linear forward-stepwise regression showed that mean DM_p was correlated with DM_0 ($r = 0.49$; $F_{1,210} = 67.58$; $P < 0.001$), T_p ($r = -0.31$; $F_{1,209} = 30.51$; $P < 0.001$), replicate ($r = -0.25$; $F_{1,208} = 22.05$; $P < 0.001$), and host sex ($r = 0.11$; $F_{1,207} = 4.40$; $P = 0.037$). These variables explained 41.6% of the variation in DM_p ($F_{1,207} = 36.79$; $P < 0.001$):

$$DM_p = 0.019DM_0 - 1.180(\text{replicate}) - 0.840T_p + 0.544(\text{host sex}) + 7.505, \quad (5)$$

with the first and second replicate coded, respectively, as 2 and 3 and male and female hosts coded, respectively, as 1 and 2.

Size-fecundity relationship in B. atlanis. Males of *B. atlanis* were larger than females in terms of hind-femur length (HFL) (males, $\bar{x} \pm SE = 1.873 \pm 0.024$ mm; $n = 74$; females, 1.706 ± 0.019 mm; $n = 68$) (Tukey's test) and DM_p (males, 5.0 ± 0.2 mg; females, 4.4 ± 0.1 mg) (Tukey's test). The two variables were positively correlated in both males ($r = 0.58$; $F_{1,72} = 36.94$; $P < 0.001$) and females ($r = 0.70$; $F_{1,66} = 65.03$; $P < 0.001$). Because the regressions of DM_p on HFL did not differ between the sexes (ANCOVA, difference between slopes [$F_{1,138} = 1.88$; $P = 0.17$] and *y*-intercepts [$F_{1,138} = 1.64$; $P = 0.20$]), the data were pooled into a single analysis ($r = 0.65$; $F_{1,140} = 100.23$; $P < 0.001$):

$$DM_p = 4.376HFL - 3.178. \quad (6)$$

The number of ovarioles in dissected females ranged from 40 to 146, with a mean of 89.2 (SE = 1.5; $n = 210$). Ovariole number and body size as measured by HFL were correlated in a non-linear fashion ($r = 0.84$; $F_{2,208} = 5878.1$; $P < 0.001$) (Figure 1):

$$\text{No. ovarioles} = 23.104HFL^{2.304}. \quad (7)$$

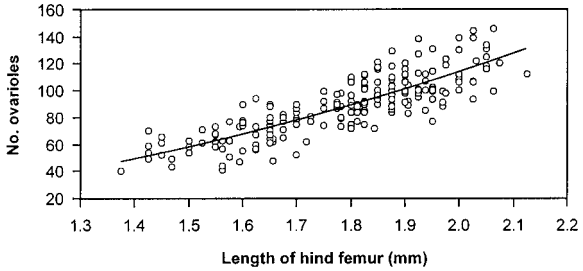

Figure 1. Relationship between the number of ovarioles and hind-femur length in *Blaesoxipha atlanis*. The regression line is described by Equation (7) (No. ovarioles = $23.104\ HFL^{2.304}$).

Females of *B. atlanis* experienced a fitness trade-off that was a function of the amount of resources available during larval development. Host sex contributed significantly to the variation in the fitness response function because the quantity of resources available to parasitoids within hosts was influenced by T_p and DM_0 (males, $r = 0.68$; $F_{5,105} = 17.81$; $P < 0.001$; females, $r = 0.53$; $F_{5,95} = 7.26$; $P < 0.001$):

$$DM_{p[\text{male host}]} = 1.465DM_0 - 1.123T_p - 0.700DM_0^2 - 0.356T_p^2 - 0.596(T_p \times DM_0) + 5.790 \quad (8a)$$

Table 3. Development of *Blaesoxipha atlanis* in males and females of *Melanoplus sanguinipes* parasitized with one, two or three parasitoid larvae. (Only data from the first of two replicates are shown; results from the second replicate were similar. See text for complete statistical analysis)

Host sex	Larvae per host	*n*	Eclosion[a] n_e (%)	Development time (h) median (range)	Dry mass[b] (mg) Total mean (SE)	Individual mean (SE)
Male	1	22	21 (95.5)	129 (105–177)	7.1 (0.4)	7.1 (0.4)
	2	20	18 (90.0)	105 (105–129)	12.8 (0.3)	6.4 (0.2)
	3	14	10 (71.4)	105	12.8 (1.1)	4.3 (0.3)
Female	1	20	19 (95.0)	129 (105–153)	8.6 (0.5)	8.6 (0.5)
	2	18	16 (88.9)	129 (105–201)	14.7 (0.7)	7.4 (0.3)
	3	14	12 (85.7)	105 (105–129)	20.4 (1.1)	6.8 (0.2)

[a] Number (% of sample n) of parasitized grasshoppers from which all sarcophagids eclosed.
[b] Total dry mass is the mass of all parasitoids developing in a host; individual dry mass is the mean dry mass of parasitoids developing in a host.

$$DM_{p[\text{female host}]} = 1.804DM_0 - 0.556T_p - 2.242DM_0^2 + 0.027T_p^2 + 0.058(T_p \times DM_0) + 7.300. \quad (8b)$$

Using ovariole number as a measure of female fitness, we estimated the predicted fitness trade-off as follows. (1) We substituted the observed minimum and maximum values of DM_0 of parasitized male (range, 66.1 − 146.6 mg) and female hosts (range, 61.1 − 225.6 mg) separately into Equations 8a and 8b, respectively, and solved for DM_p for different numbers of larvae (T_p = 1, 2,. . ., 6). (2) We solved Equation 6 for HFL and substituted this function into Equation 7. (3) We substituted the results from step 1 into the equation from step 2 and solved for the number of ovarioles. Figure 2 shows the relationship between the number of ovarioles and the number of larvae developing in male and female grasshoppers differing in body size. Parasitoid fitness will be constrained less by gregarious development if larvae develop in females, rather than males, of *M. sanguinipes.*

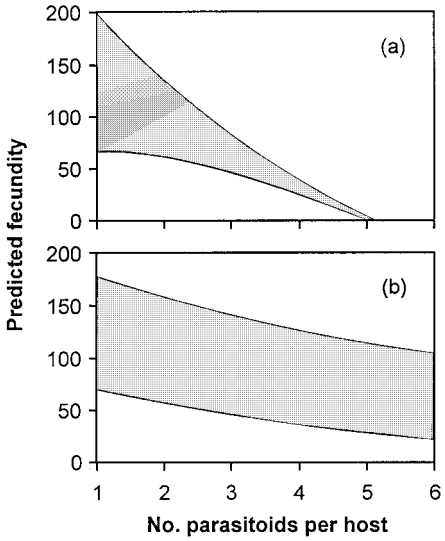

Figure 2. Theoretical fitness trade-offs in females of *Blaesoxipha atlanis* between fecundity as measured by ovariole number and numbers of larvae developing in males (a) and females (b) of *Melanoplus sanguinipes* differing in size. Shaded areas were obtained by extrapolation from experiments in which the number of parasitoid larvae per host did not exceed three (see text for details). The upper and lower boundaries of the areas occur at the maximum and minimum values, respectively, of host dry mass at parasitization (males, 66.1–146.6 mg; females, 61.1–225.6 mg).

Intensity of parasitism by B. atlanis *in the field.* Field-collected males ($\bar{x} \pm SE = 1.966 \pm 0.031$ mm; n = 42) and females (1.844 ± 0.029 mm; n = 26) did not differ in body size as measured by HTL from counterparts reared in the laboratory as solitary larvae in *M. sanguinipes* (males, 1.934 ± 0.046 mm; n = 20; females, 1.786 ± 0.029 mm; n = 14] (Kolmogorov–Smirnov two-sample test: males, D = 0.195; $D_{0.05}$ = 0.369; females, D = 0.308; $D_{0.05}$ = 0.450). Both sexes were significantly larger than laboratory-reared individuals developing in double- and triple-parasitized hosts (males, 1.851 ± 0.028 mm; n = 54; D = 0.325 [$D_{0.05}$ = 0.297]; females, 1.686 ± 0.021 mm; n = 54; D = 0.434 [$D_{0.05}$ = 0.324]).

Discussion

Larvae of the sarcophagid fly *B. atlanis* can successfully develop either as solitary or gregarious endoparasitoids in their natural grasshopper host, *M. sanguinipes*. Host survival and parasitoid growth were influenced by the number of larvae present in each grasshopper, however. Parasitized grasshoppers died earlier than their unparasitized counterparts. The amount of lifeloss, a measure of survival time lost due to parasitism, was similar in male and female hosts containing one parasitoid larva. Lifeloss increased with the number of larvae and was greater in parasitoids developing in male than female hosts, which suggests that gregarious development was under host sex-specific constraints. In male *M. sanguinipes*, parasitoid development and growth appeared to be limited mainly by the amount of resources available at parasitization, i.e., initial host size (Table 1). Although adult females were larger than males in terms of DM, increased size by itself could not explain qualitative differences between the host sexes. Parasitoid demands for nutritional resources compete with host demands for gonadal development, with females representing a distinctly different physiology to the parasitoid. Compared with males, females of *M. sanguinipes* have a greater capacity to assimilate dry mass and to supply metabolites for gonadal development, but they also allocated a relatively greater proportion of biomass to gonadal than somatic tissues (T. Danyk, unpubl.). Whereas the percentage of total DM allocated to gonadal tissues reached a plateau at 5% in unmated males 6 days after ecdysis, it remained constant at 3% for 10 days after ecdysis in unmated females, increasing to 14% thereafter. Miranpuri et al. (1991) reported that the concentration of protein and carbohydrate was less in the hemolymph of males than females, varying up to four-fold with the stage of oocyte development in the latter (Elliott & Gillott, 1977). Host age was also shown to affect parasitoid growth and development in other systems, either because host resources were depleted (Sandlan, 1982) or became unavailable due to host development (Otto & Mackauer, 1998). It is possible that resources allocated to oocyte development in female hosts are less readily available to the parasitoid than resources allocated to spermatocyte development in male hosts.

The development time and adult size of *B. atlanis* were influenced by the number of larvae present in each host. Solitary larvae developed for longer, and were on average larger as adults, than counterparts developing gregariously (Table 3). A decrease in development time with an increase in parasitoid load was also observed in a braconid wasp (Beckage & Riddiford, 1978, 1983) and in several non-parasitic sarcophagid flies (Baxter & Morrison, 1983; So & Dudgeon, 1989). The decline in body size was especially pronounced in cases of three larvae developing gregariously in male grasshoppers. Gregarious development incurred a fitness cost due to the positive correlation between body size and fecundity in females (Figures 1 and 2) and, possibly also, in males. A reduction in body size and fecundity as a result of competition between gregarious larvae is a common phenomenon in parasitic sarcophagids (Coupland & Baker, 1994) and tachinids (King et al., 1976; Grenier, 1981), non-parasitic sarcophagids (Baxter & Morrison, 1983; So & Dudgeon, 1989; Tanaka et al., 1990), and hymenopteran parasitoids (Rojas-Rousse et al., 1988; Taylor, 1988; Reitz & Adler, 1995).

Parasitoids generally kill their hosts. However, parasitized *M. sanguinipes* often survived parasitism by *B. atlanis*, with one exception: all male grasshoppers containing two or three larvae died (Table 1). English-Loeb et al. (1990) also observed that approximately 25% of arctiid larvae parasitized by *Thelairia bryanti*, a gregarious tachinid, survived parasitism. King et al. (1976) reported that larval mortality in the sugarcane borer, *Diatraea saccharalis*, parasitized by the tachinid *Lixophaga diatraeae* declined with an increase in host instar and body size. We suggest that parasitoid larvae function as a metabolic sink, analogous to gonadal tissue. The increased capacity of female acridids to supply the gonads with resources may explain why female hosts lived longer and were more likely to survive parasitism than their male counterparts.

In conclusion, our experiments show that more than one larva of *B. atlanis* can successfully develop in *M. sanguinipes*. Gregarious development, however, results in a significant cost to the parasitoid in that adults are smaller and, in females, have reduced fitness. Compared with healthy grasshoppers, parasitized individuals feed less (T. Danyk, unpubl.) and are therefore unable to replenish food reserves quickly. Unlike solitary parasitoids in which supernumerary larvae are being eliminated by physical combat or physiological suppression (Mackauer, 1990), larvae of *B. atlanis* compete for resources. Reduced survival is a consequence of the host either being initially small or being unable to satisfy the increasing nutritional demands of the developing parasitoids. Body size did not differ between field-collected parasitoids and individ-

uals developing singly in *M. sanguinipes* in the laboratory, which indicates that gregarious development is uncommon. Facultative gregarious development in *B. atlanis* may be adaptive, however. The reduction in female fitness due to small body size is probably less than the fitness gain due to more than one offspring surviving in each host. In the laboratory, 98% of maggots failed to develop successfully into adults when placed directly on a healthy grasshopper (T. Danyk, unpubl.). If larval survival is equally low in the field, the potential for solitary and gregarious development would contribute to the maintenance of *B. atlanis* populations, especially during periods when few hosts are available.

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