Effect of host quality of *Callosobruchus maculatus* (Coleoptera: Bruchidae) on performance of the egg parasitoid *Uscana lariophaga* (Hymenoptera: Trichogrammatidae)

J. Spitzen and A. van Huis*

Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH Wageningen, The Netherlands

Abstract

Development and reproductive success of the solitary egg parasitoid *Uscana lariophaga* Steffan were examined after development in eggs of the bruchid storage pest *Callosobruchus maculatus* Fabricius reared at either low or high densities on cowpea seeds and laid at day 1 and 4 of maternal life. Both bruchid larval competition and maternal age negatively affected egg size, but the latter more than the former. *Uscana lariophaga* reared in small hosts developed slower, were smaller and produced fewer eggs compared to parasitoids reared in large hosts. Fecundity of the parasitoid was heavily influenced by host egg size. This was reflected in the values for the intrinsic rate of increase of *U. lariophaga*, which differed for wasps that developed in host eggs laid by bruchid females of different age. Wasps allocated marginally more female offspring to larger hosts.

Keywords: *Callosobruchus maculatus*, *Uscana lariophaga*, *Vigna unguiculata*, Coleoptera, Bruchidae, Hymenoptera, Trichogrammatidae, cowpea, competition, maternal age, egg size, fitness

Introduction

*Uscana lariophaga* (Steffan) (Hymenoptera: Trichogrammatidae) is an egg parasitoid of *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae), a serious pest of stored cowpea *Vigna unguiculata* (L.) Walp. (Fabaceae) in West Africa (van Huis et al., 1990; van Alebeek, 1996; Stolk et al., 2001).

A large amount of variation in egg size is present within populations of the seed beetle, *C. maculatus* (Fox, 1993, 1994). This variation results from both phenotypic variation amongst females, in which larger females lay larger eggs (Fox, 1993), as well as within-female variation due to maternal age effects, older females laying smaller eggs (Wasserman & Asami, 1985; Fox, 1993). Egg size in bruchids can also vary by means of competition, but previous studies have indicated that such an effect is small (Kawecki, 1995; Fox & Savalli, 1998). Egg size is the most widely used predictor of egg quality, under the assumption that large eggs produce offspring with higher fitness. Azevedo et al. (1997) reviewed 137 studies and found that 63% of these indicated positive associations between egg size and components of offspring fitness. The remainder of the studies indicated that egg size does not fully predict offspring fitness.

The subject of this study was not the effect of egg size on the fitness of *C. maculatus*, but the effect on the fitness of its egg parasitoid *U. lariophaga*. In *Trichogramma* egg parasitoids, host size influences the quality of offspring and their suitability as biological control agents (Marston & Ertle, 1973; Navarajan et al., 1981; Bai et al., 1992; Greenberg et al., 1998; Luck & Forster, 2003). *Trichogramma* spp. are gregarious parasitoids, and the female can deposit a variable number of eggs into each host depending on several factors, which include host size, shape, age and chemical content.
sex allocation. Eggs of different sizes and if this would have an effect on parasitoid would be able to discriminate between host tests were also conducted to investigate whether the female size of the host egg or by the nutritional quality when she her offspring was triggered by the results of measuring the size of the host egg or by the nutritional quality when she probed the host egg with her ovipositor.

In this study, it was examined whether the performance of the egg parasitoid is affected when developing in different sized host eggs, obtained by rearing the bruchid host at high and low densities and by using bruchid females of young and advanced age. This was studied by using one leguminous species (cowpea), chickpea and pigeonpea. However, it was not clear whether the decision of the female wasp when allocating the sex of her offspring was triggered by the results of measuring the size of the host egg or by the nutritional quality when she probed the host egg with her ovipositor.

Materials and methods

The Campinas strain of *C. maculatus* obtained from Brazil in 1975 and maintained continuously in culture since then at Royal Holloway College (University of London) was used in this study. In September 2002, a sample of this culture was brought to Wageningen University where the bruchids were maintained. From the Niamey region in Niger where it was collected in December 1990.

Bruchid egg size variation

Adults of *C. maculatus* were reared at low and high densities. The low density bruchid treatment was obtained by putting 25 pairs of adults on 1250 seeds as a monolayer in a glass Petri dish (diameter 18 cm, height 4 cm) for 24 h. Ten seeds with one bruchid egg were selected and put in glass tubes (length 6.7 cm, diameter 1.7 cm). The high density bruchid treatment was obtained by putting 250 pairs of adults on 50 beans for 24 h. Seeds containing more than 20 eggs were selected and incubated individually in similar-sized glass tubes. After 20 days incubation, tubes were checked daily, and the emerged bruchids were sexed and removed. On the third day, after the first appearance of adults, a random selection of freshly emerged females was weighed individually to the nearest 0.001 mg using a Cahn C-33 automatic electro balance (Ankersmit, The Netherlands). Each female with a male of corresponding age and treatment was placed in a 9 mm Petri dish with ten seeds. Seeds were replaced daily until the fourth day. Two eggs per pair, laid on the first and fourth day, were measured using a stereomicroscope equipped with an ocular micrometer (accuracy ± 0.015 mm). A mobile table was used in order to arrange the eggs on the seeds in a horizontal position and then egg length and width were measured. The egg size was assumed to be half a prolate spheroid and its volume (*V*) was calculated from the width (*w*) and length (*l*) using the formula, \( V = \frac{1}{6} \pi w^2 l_{1/2} \). Female body size, by means of elytron length, was measured post mortem (accuracy ± 0.05 mm).

Host egg volume and fitness of *U. lariophaga*

Adult wasps of *U. lariophaga* less than 20 h old were allowed to mate for 1 h in glass tubes. Females were then selected and offered five host eggs less than 24 h old from either low or high-density reared bruchids. This procedure was followed for eggs laid by 1-day-old bruchids and three days later with eggs laid by 4-day-old female bruchids. In order to check whether superparasitism occurred, the process of parasitization was observed through a stereomicroscope. In cases where superparasitism occurred, these eggs were removed from the experiment. Females were removed if no oviposition was observed for at least 5 min. Parasitized eggs were incubated individually in glass tubes (5.5 x 1.3 cm) at 25 ± 1°C. Successfully parasitized eggs turned brown or black and were counted. Development time of *U. lariophaga* was measured by counting the number of emerged wasps, twice daily. Parasitoids were sexed and emerged females were placed together with a male and each offered 30 'normal sized' host eggs, daily. To avoid damaging the wasps, seeds with hosts were placed on a punched piece of cardboard and placed in a glass tube (9.5 x 2.2 cm). Fecundity of these females was measured daily using the same method as described earlier. Longevity was checked twice daily and adult size by means of hind tibia length (accuracy ± 0.005 mm) was measured post mortem. The intrinsic rate of increase of the wasp population per day, \( r_{na} \), was calculated according to Howe’s (1953) simplified method: \( r_{na} = \ln (x/(t + 0.5p)) \) where *x* is the number of female offspring that emerged per female parent, *t* is the development period in days, and *p* is the longevity of the female parent (oviposition period).

Host egg size and sex allocation by *U. lariophaga*

A dual choice-experiment was carried out to investigate if *U. lariophaga* females would alter the sex ratio of their offspring when offered eggs of different size. Wasp were offered two egg size classes: large eggs obtained from 1-day-old low-density reared bruchids, and small eggs from 4-day-old high-density reared female bruchids. To verify whether different egg sizes were obtained, a random sample was taken from each group and measured. The hosts from both
groups were not older than 24 h when offered to the wasps. Seeds containing either a small or a large egg were marked with a Lumocolor pen accordingly. A pair of wasps (≤24 h old) was placed in a tube with 34 host eggs, 17 host eggs from each group. After 24 h, eggs from the different treatments were separated and incubated. The sex ratio was scored when wasps emerged.

Analyses

Differences in bruchid development time, adult bruchid weight and elytron length as a result of the two rearing methods (see above) were tested with an independent-sample T-test, differences in weight and elytron length with a Cochran C test, and differences between egg size with T-tests for two independent samples. The effects of the bruchid rearing methods on wasps’ fitness parameters were conducted with T-tests for two independent samples. Mortality and sex ratio differences of the parasitoids were analysed by a Chi-square test. The relationship between hind tibia length and longevity or fecundity of the wasps was tested with a Pearson correlation. Sex allocation differences were tested with a paired-sample T-test. The two host groups used for this experiment were tested for differences in egg volume with an independent-sample T-test.

Results

Bruchid egg size variation

Individuals of C. maculatus reared under low larval densities developed about 1 day faster than those reared at high larval densities (T-test, \( P < 0.001 \); table 1). The sex ratio of the bruchids developing under low densities was similar to that of bruchids reared at high densities (54 and 51%, respectively). Female bruchids reared under low larval densities were significantly heavier and larger based on elytron length than those reared at high larval densities (fig. 1, table 2). There was a positive linear relationship between elytron length and fresh weight of female bruchids reared at the two densities (fig. 1). The variances of both elytron length and weight of female bruchids reared under high larval densities developed about 1 day faster than those reared at high larval densities (table 3). Although the sex ratio of wasps that developed on eggs laid by 1-day-old females was higher than that from eggs laid by 4-day-old females, the difference was not significant. The development time of wasps was shorter in the largest compared to the smallest eggs (largest laid by 1-day-old females reared at low larval densities, and smallest laid by 4-day-old females reared at high larval densities) (fig. 2b). Most wasps appeared to emerge or die during the night, and because of their short longevity (1–4 days), differences in development time and longevity were difficult to detect. However, it appeared that the parasitoids lived for a shorter period when developing in smaller eggs (weakly significant different; \( P < 0.1 \), fig. 2c). The fecundity of U. lariophaga was higher in eggs laid by bruchid females on the first day compared to those laid on the fourth day of maternal life (T-test for all host eggs laid on days 1 and 4; \( P < 0.001 \)) (fig. 2d). The size of U. lariophaga (expressed as hind tibia length) reared in the largest eggs was larger than when reared in the smallest eggs (fig. 2e). Wasp size was also positively correlated with both the wasps’ longevity and fecundity (longevity: \( y = 23.73x + 0.15 \), \( R^2 = 0.110 \), \( n = 22 \); fecundity: \( y = 499.9x – 16.2 \), \( R^2 = 0.035 \), \( n = 28 \)), although the Pearson correlations were weakly

<table>
<thead>
<tr>
<th>Larval density</th>
<th>n</th>
<th>Development time (days)</th>
<th>Female (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>152</td>
<td>26.8 ± 1.64 a</td>
<td>53.9</td>
</tr>
<tr>
<td>High</td>
<td>234</td>
<td>27.9 ± 1.65 b</td>
<td>51.3</td>
</tr>
</tbody>
</table>

Means in column followed by a different letter are significantly different (\( P < 0.001 \), T-test).

Table 2. Mean fresh weight and elytron length of female Callosobruchus maculatus reared at low and high densities.

<table>
<thead>
<tr>
<th>Larval density</th>
<th>n</th>
<th>Weight (mg)</th>
<th>Elytron length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>20</td>
<td>5.93 ± 0.51 a</td>
<td>2.16 ± 0.01 a</td>
</tr>
<tr>
<td>High</td>
<td>27</td>
<td>3.78 ± 0.58 b</td>
<td>1.83 ± 0.02 b</td>
</tr>
</tbody>
</table>

Means in columns followed by different letters are significantly different (\( P < 0.001 \), T-test).
Fig. 2. Effect of rearing density and maternal age of *Callosobruchus maculatus* on egg volume (a) and effect of host size on fitness parameters of *Uscana lariophaga* (b–e) (means ± SD): development time (b), longevity (c), fecundity (d) and hind tibia length (e). L 1 and L 4: low density reared bruchids; maternal age day 1 and 4, respectively. H 1 and H 4: high density reared bruchids; maternal age day 1 and 4, respectively. HTL = hind tibia length. Means within each histogram with different letters are significantly different (Mann Whitney U: *P* < 0.05, *P* < 0.1).
significant ($P = 0.069$) and not significant ($P = 0.163$) respectively. The intrinsic rate of increase of *U. lariophaga* reared from hosts reared under low and high density differed by only 6%, while those reared from eggs laid on the first and fourth day of maternal life differed by 13% (table 4).

**Host egg size and sex allocation by *U. lariophaga***

Eggs obtained from 1-day-old female bruchids reared at low larval densities and those obtained from 4-day-old female bruchids reared at high larval densities differed significantly in volume (table 5). Larval mortality of *U. lariophaga* did not differ between these host groups. Mothers allocated relatively more females to larger hosts, but not significantly.

**Discussion**

**Bruchid egg size variation**

Larvae of bruchids develop internally and do not move from one seed to another and are therefore food-limited. Under high larval competition, *C. maculatus* adults that emerged had a reduced body size with a lower weight, which is in accordance with results obtained by Credland et al. (1986), Colegrave (1993), Wilson (1994), Kawecki (1995) and Fox & Savalli (1998). Beetles that develop under high density conditions resulted in individuals with reduced body size which produced smaller eggs. Fox & Savalli (1998) also showed that *C. maculatus* reared at higher densities produced smaller eggs. Another effect of high larval competition was an extended egg-to-adult development period. Only Fox et al. (1999) working with *Stator pruininus* Horn (Coleoptera: Bruchidae) found some evidence of longer development with increased larval competition at the highest density of 20 eggs per seed of *Acacia greggii* Gray (Leguminosae: Fabaceae). The significant egg size response to larval competition was probably because very high densities were used, and the strain 'Campinas' appears to be very sensitive to competition (Credland et al., 1986). In the present study, maternal age had a greater effect on egg size than competition. The resources of females gradually diminish with maternal age, and with it offspring quality, which is apparent through a reduction in egg size (Fox, 1993). Another factor which has a positive effect on egg size is the provisioning of sperm by the male, viz. obtaining multiple or larger ejaculates during female adulthood (Fox, 1993; Kawecki, 1995; Fox & Savalli, 1998). Aspects of sperm competition and ejaculate size were not included in this study as freshly emerged adult beetles were mated in pairs. Males were taken from the same rearing densities as females to minimize the effects of ejaculate size.

In Fox & Savalli’s studies (1998), egg size differences obtained by rearing bruchids under different densities did not exceed 10%, but they only measured eggs that were laid within the first 24 h of adult life. To determine effects of host egg size on the egg parasitoid *U. lariophaga*, the effects of both maternal age and larval competition were combined in order to obtain size differences of up to 18%.

**Host egg volume and fitness of *U. lariophaga***

The effects of smaller host eggs of *C. maculatus* on the performance of *U. lariophaga* were: (i) longer egg-to-adult development periods; (ii) reduced body size; (iii) shorter lifespan; and (iv) reduced fecundity of the adult wasp. The strongest effect was on the fecundity of the wasp and this was mainly the result of host eggs derived from beetles of

<table>
<thead>
<tr>
<th>Larval density</th>
<th>Maternal age (days)</th>
<th>Egg volume ($\mu$m$^3$)</th>
<th><em>U. lariophaga</em> mortality (%)</th>
<th>Females (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>1</td>
<td>19.8 ± 2.4 (15) $a$</td>
<td>16.9 (12) $a$</td>
<td>74.2 ± 17.7 (12) $a$</td>
</tr>
<tr>
<td>High</td>
<td>4</td>
<td>16.2 ± 3.1 (15) $b$</td>
<td>21.5 (12) $a$</td>
<td>67.7 ± 18.6 (12) $a$</td>
</tr>
</tbody>
</table>

Results of host parasitization by *U. lariophaga*: parasitoids’ larval mortality (weighted mean ± SD, depending on number of parasitized eggs per wasp), and sex ratio (± SD) of the emerging wasps (replicates in brackets). Means in columns followed by a different letter are significantly different: $^1P < 0.001$, T-test; $^2P = 0.45$, Chi square; $^3P = 0.30$, paired sample T-test.
different maternal age. Judging from the intrinsic rate of increase of the wasps, the effect of host maternal age on the potential population increase of the wasp was larger than the effect of density. The effect of maternal age of the host is probably due not only to a smaller size but also to a lower nutritional quality of the egg in older females. Fox (1993) also suggested that host eggs laid by older beetles may be of poorer quality than those laid by young females. The lower host quality will then be the cause that wasps perform less well as expressed by their lower intrinsic rate of increase. It could be that the quantitative variations in host resources, as expressed in host size, are less important than the nutritional quality of the egg content.

Host egg size and sex allocation by U. lariophaga

Physical cues such as size, shape, curvature and colour of the host eggs are considered to be important criteria used by Trichogramma species for host selection (Salt, 1935; de Jong & Pak, 1984; Schmidt & Smith, 1987). In addition to physical cues, chemosensory information may be collected by the parasitoid as she examines an egg before oviposition, i.e. by drumming and examining the surface of the egg with her antennae (Salt, 1937; Klomp et al., 1980; de Jong & Pak, 1984; Stolk, 2002, page 97). Van Huis & de Rooy (1998) found sex ratio differences of U. lariophaga reared from different host sizes. These host sizes, with volume differences up to 8%, were obtained by rearing C. maculatus on three different hosts (cowpea, chickpea and pigeonpea). The authors suggested that offspring sex allocation was triggered by the wasp measuring the size of the host egg (Klomp & Teerink, 1962; Schmidt & Smith, 1986, 1989) or by the wasp determining the nutritional quality of the host during ovipositor insertion preceding oviposition. In several studies on egg parasitoids (mainly Trichogramma spp.) the effect of different egg sizes on sex allocation and that of different host species are confounded (Klomp & Teerink, 1962; Marston & Ertle, 1973; de Jong & Pak, 1984; King, 1989; Bai et al., 1992; Greenberg et al., 1998). In the present study, only one leguminous plant species (cowpea) was used and egg size differences up to 18% were recorded. Although marginally more females were produced in larger eggs, this effect was not significant. This suggests that infochemical cues are probably more important for the wasp when allocating either a male or a female egg than host size.

Acknowledgements

The authors wish to thank J.C. van Lenteren and J.A. Harvey for reviewing earlier drafts of this manuscript. We would like to thank P.F. Credland for sending us the Campinas strain of the bruchid. F. van Aggelen, L. Koopman and A. Gidding are acknowledged for maintaining the insect colonies. Discussions about the set-up of the experiments with E.I. Niyibigira were appreciated.

References


Effect of host quality on Uscana lariophaga


(Accepted 25 February 2005) © CAB International, 2005