

This is an electronic version of the article published as

King, B. H. 2000. Sex ratio and oviposition responses to host age and the fitness consequences to mother and offspring in the parasitoid wasp *Spalangia endius*. Behavioural Ecology and Sociobiology 48:316-320. The original publication is available at www.springerlink.com.

Sex ratio and oviposition responses to host age and the fitness consequences to mother and offspring in the parasitoid wasp *Spalangia endius*

B. H. King

Department of Biological Sciences, Northern Illinois University,

DeKalb, IL 60115, USA

E-mail: bking@niu.edu

Abstract

In the parasitoid wasp *Spalangia endius* more offspring and a greater proportion of daughters were oviposited in, and emerged from, 0-day-old versus 3-day-old hosts. Offspring that developed on the younger hosts 1) were larger at adulthood, 2) developed more quickly, 3) had higher survivorship to adulthood, and 4) were more often able to chew their way out of the host. Sons and daughters did not differ in how host age affected their size, development rate, or survivorship. The greater proportion of daughters from the younger hosts may be adaptive as described by the host quality model (a variant of the Trivers and Willard hypothesis). It is adaptive if greater size or more rapid development has a more positive effect on daughter's than son's fitness and the positive effect is large enough to compensate for sons being trapped disproportionately to daughters in the older hosts. Despite greater success at drilling the younger hosts, mothers did not try to drill them sooner or more often. Having previously oviposited on the older hosts rather than the younger hosts had no detrimental effect on the mother's subsequent longevity or offspring production.

Key words Sex Ratio · Host age · Parasitoid wasp · Offspring fitness · Development time

Introduction

How many and what sex offspring a mother produces may depend on the availability of resources for her offspring (Charnov 1982). When resource quality or quantity is high, females should preferentially oviposit the sex more positively affected, an idea called the host quality model when applied to parasitoids (Charnov et al. 1981). Here, using the parasitoid wasp Spalangia endius, I examine maternal choice of host age in relation to the host quality model and effects of choice not just on the offspring's fitness, but also on the mother's subsequent fitness, a seldom explored area (Godfray 1994). The host quality model is becoming widely accepted (e.g., Mousseau and Fox 1998); but in studies of Spalangia, support has been mixed (e.g., King and King 1994; King and Lee 1994; Napoleon and King 1999).

Spalangia parasitize pupae of certain fly species (Rueda and Axtell 1985). The age of hosts in a given patch is variable (personal observation). A female drills through the puparium (case) of a host with her ovipositor (Gerling and Legner 1968). She then deposits an egg on the host pupa within or feeds on exuding host fluids, which are necessary for continued egg production. The parasitoid larva bites through the host pupal integument (within the puparium) and feeds on the host fluids; once adult, the parasitoid chews out through the puparium. S. endius is a solitary parasitoid, i.e., one offspring completes development per host. Older host pupae weigh less than younger ones (King 1990); and part of the biomass has been converted to nonliquid adult body parts, which Spalangia cannot ingest (Gerling and Legner 1968).

Here I test the host quality model prediction that mothers oviposit a greater proportion of daughters on 0-day-old versus 3-day-old hosts. A greater proportion of daughters emerging from the younger hosts is a result of the mother's differential oviposition of the sexes, provided differential mortality of the sexes during development can be ruled out. I also test the host quality model assumption that developing on a 0-day-old host has a more positive effect on the fitness of a daughter than a son. I use three common fitness measures, size, survival, and rate of development, and one previously unexplored measure, ability of offspring to exit the host. Finally I examine the timing of host age choice and the cost to the mother in terms of her own offspring production and longevity.

Methods A Florida strain of S. endius was maintained at 23-28°C, 24 h light, using Musca domestica as hosts. Experiments were performed within 6 mo (less than 8 generations) of collection of this strain, except the second subsequent fitness experiment and the second video experiment, which were performed a year later.

Hosts were produced following King (1998). "0-day-old hosts" were 0 - 24 h old (from when the puparium turned red) when initially presented to the parasitoids; "3-day-old hosts" were 3 d older. Parasitoids were less than 2 d old at mating and used in an experiment within 1 d of mating. I presented hosts to females in plastic vials (40 mm high by 36 mm top diameter by 27 mm bottom diameter) with a drop of honey for food. A new set of hosts was presented each day.

I present means \pm 1 SE, range. "2t P" denotes a two-tailed P value, "1t P" a one-tailed value.

Offspring production, sex ratio and size I presented each of 39 mated females with 10 0-day-old and 10 3-day-old hosts simultaneously on each of 2 successive days. Females moved freely across the ridge of used fly media that separated the two host age groups. No female successfully parasitized all hosts of a given host age class (as evidenced by the emergence of at least 1 adult host). The dry weight of one adult offspring of each sex from each female was measured to the nearest 0.001 mg.

I analysed number of adult offspring by repeated measures ANOVA with host age and day as within-subject factors. I tested effects of host age and day on offspring sex ratio by loglinear analysis. I analysed offspring weight by repeated measures ANOVA with host age and offspring sex as within-subject factors (day was nonsignificant and so excluded).

Offspring survivorship Greater mortality of daughters than sons can be detected by greater mortality of mated females' offspring (sons and daughters) than of virgin females' offspring (sons) (Sandlan 1979). A virgin or mated female was given 20 0-day-old or 20 3-day-old hosts for 1 d. Ten hosts were dissected and the eggs were counted; the other 10 were allowed to complete development and the adult offspring were counted. Survivorship was estimated as number of adult offspring divided by number of eggs. I tested effects of host age and mating status on survivorship by loglinear analysis. I analysed number of eggs by repeated measures ANOVA with mating status and host age as within-subject (i.e., within replicate) factors.

Duration of offspring development

A petri dish of 0-day-old hosts and a dish of 3-day-old hosts were placed in separate containers full of adult parasitoids for 4 h at 27°C and then allowed to develop at 20-24°C. After adult flies emerged, I isolated remaining hosts individually in test tubes and checked daily for parasitoid emergence. The parasitization duration and temperatures were chosen to reduce treatment differences in time of initial parasitization and to lengthen development and thus magnify any difference between treatments. This experiment was repeated thrice, but there were no repetition effects. Thus, repetitions were combined and a simple two-way ANOVA of the effects of host age and offspring sex on duration of development is presented.

Mother's subsequent fitness

In the first subsequent fitness experiment I divided 28 females equally into two treatments: 6 d of experience with 20 0-day-old or 20 3-day-old hosts, followed by 20 3-day-old hosts on the seventh day. Thereafter each female was kept in a test tube with a moistened cotton plug until she died because ability to survive until she finds hosts may contribute to a female's lifetime fitness. The effect of previous host age experience was analysed by a paired t-test for longevity and by a sign test for offspring production on the seventh day (host age treatments paired within each replicate).

The second subsequent fitness experiment used 4 d of experience followed by 10 0-day-old and 10 3-day-old hosts on day 5. The effect of previous host age experience on offspring production on day 5 was analysed by repeated measures ANOVA with replicate as a blocking effect, current host age as a within-subject factor

within mother and previous host age as a between-subject factor.

Drilling and preferenceIn the inexperienced female video-experiment, I determined whether 3-day-old hosts are more difficult to drill into and less often chosen than 0-day-old hosts. I videotaped each of 40 mated females that had no prior experience with hosts. Each was videotaped for 3 h in a 35 mm diameter X 10 mm deep petri dish with one 0-day-old and one 3-day-old host. The hosts were parallel to each other, about 2.5 parasitoid lengths apart, and secured with a small drop of water. I alternated which age of host was in the left position. I subsequently isolated the hosts in test tubes to allow parasitoid offspring to complete development.

During videotaping, illumination was from fibre-optic lights, which gave off no noticeable heat. I recorded which host the female first contacted (usually with unbent antennae), first antennated (tapped with antennae bent less than 90° at their elbows) and first attempted to drill for at least 1 min (King 1994). For each host age, I recorded number of drill attempts of more than 1 min, proportion that were completed and their duration, and whether the female subsequently fed at the site.

In the experienced female video-experiment, I determined whether females preferentially drill 0-day-old hosts before 3-day-old hosts. The procedure was as in the previous video experiment except that prior to videotaping, I gave each female (N = 29) 10 0-day-old and 10 3-day-old hosts for 1 d. From these tapes I collected data on which host the female first contacted, first antennated and first attempted to drill.

Results

Offspring production, sex, and size

Mothers produced more adult offspring from 0-day-old versus 3-day-old hosts (Table 1) and a lower proportion of sons (17% versus 26% on day 1, 8% versus 22% on day 2; $G = 12.03$, $P = 0.0005$), but the decrease in proportion of sons was not significantly dependent on day ($G = 1.70$, $P = 0.19$). Significance of the sex ratio by day interaction was marginal ($G = 3.76$, $P = 0.052$).

Offspring from the younger hosts were larger, and the degree to which they were larger was not significantly different for daughters versus sons (Table 2).

Offspring survivorship

More eggs were oviposited by females that received 0-day-old versus 3-day-old hosts and by mated versus virgin females (Table 3). Offspring survivorship was greater on 0-day-old versus 3-day-old hosts (0.80 versus 0.05 for virgin females, 0.68 versus 0.06 for mated females; $G = 170.4$, $P < 0.0001$), and this effect of host age on survivorship was independent of mating status ($G = 0.34$, $P = 0.56$). Survivorship was independent of maternal mating status ($G = 0.83$, $P = 0.36$).

Mated females produced a greater proportion of sons when they received only 3-day-old versus only 0-day-old hosts (0.42 ± 0.15 , 0.00 - 1.00, N = 8 versus 0.15 ± 0.03 , 0.00 - 0.60, N = 32; Mann-Whitney U = 81.5, 1t $P = 0.05$).

Offspring developmentFemales and males took equally longer developing on 3-day-old versus 0-day-old hosts, about 1 d longer; and males developed more quickly than

females (Table 4).

Hosts lacking emergence holes were dissected open two weeks after the last parasitoid emerged. Nineteen unemerged adult parasitoids were found; all but 1 were dead or nearly so. The percent of adult parasitoids that were unemerged was significantly greater for 3-day-old hosts than for 0-day-old hosts (8.51% versus 2.93%, $G = 5.57$, $P < 0.03$). For 0-day-old hosts there was no significant difference in the proportion of sons among unemerged versus emerged parasitoids (43%, $N = 7$ versus 31%, $N = 230$; $G = 0.40$, $P > 0.50$). For 3-day-old hosts, there was a significantly higher proportion of sons among unemerged versus emerged parasitoids (83%, $N = 12$ versus 29%, $N = 128$; $G = 13.93$, $P < 0.001$).

Subsequent fitness

In the first subsequent-fitness experiment, prior exposure to 0-day-old versus 3-day-old hosts had no significant effect on a mother's offspring production on day 7 (sign test: 2t $P = 0.50$) or on her subsequent longevity (9.4 ± 0.4 d versus 10.1 ± 0.3 d; $t_{13} = 1.43$, 2t $P = 0.11$). Zero of 14 females in the 3-day-old host treatment and 2 of 14 females in the 0-day-old host treatment produced offspring on day 7.

In the second subsequent-fitness experiment, most mothers still produced offspring on their fifth day of oviposition: 14 of 23 mothers that received 0-day-old hosts; 15 of 23 mothers that received 3-day-old hosts. Whether a female had previously been exposed to 0-day-old or 3-day-old hosts had no significant effect on the number of offspring she produced on the fifth day (ANOVA: $F_{1,22} = 0.13$, $P = 0.72$), and there was no significant interaction between the effect of previous host age (days 1-4) and current host age (day 5) (ANOVA: $F_{1,22} = 0.94$, $P = 0.34$).

Drilling and preference

Females exhibited no strong preference between 0-day-old hosts and 3-day-old hosts prior to their first videotaped drilling attempt, regardless of whether they had been exposed to hosts the previous day. For inexperienced females, the number of females choosing the 0-day-old host versus the 3-day-old host did not differ for first drill attempt (19 versus 14; $X^2 = 0.76$, $P = 0.38$), first host contact (24 versus 15; $X^2 = 2.08$, $P = 0.15$), or first antennating (20 versus 18; $X^2 = 0.11$, $P = 0.75$). The number of drill attempts per female in 0-day-old hosts was not significantly greater than in 3-day-old hosts (1.97 ± 0.27 versus 2.39 ± 0.39 ; $t_{37} = 0.92$, 2t $P = 0.36$) even if females that host fed are excluded. Likewise, for experienced females, the number of females choosing the young versus old host also did not differ for first drill attempt (13 versus 13; $X^2 = 0.00$, $P = 1.00$), first host contact (12 versus 16, $X^2 = 0.57$, $P = 0.45$), or first antennating (13 versus 15; $X^2 = 0.14$, $P = 0.71$).

For inexperienced females, drill attempts were more often completed on 0-day-old than on 3-day-old hosts ($55\% \pm 9\%$ versus $18\% \pm 7\%$; $t_{16} = 3.23$, 1t $P = 0.005$). However, there was no evidence that completed drills were quicker on 0-day-old than on 3-day-old hosts (within-female comparison: 18.8 ± 4.8 min versus 13.6 ± 0.8 , $t_4 = 1.08$, 2t $P = 0.34$; treating each drill as independent: 16.7 ± 2.0 min versus 12.6 ± 1.0 ; $t_{24,13} = 1.81$, 2t $P = 0.08$).

Nine of 33 completed drills resulted in host feeding: 4 on 0-day-old hosts, 5

on 3-day-old hosts. No adult parasitoid offspring nor adult flies were produced from these 9 hosts. Four of the 9 females that host fed completed drills on both hosts; in all cases, the female fed from the 3-day-old host before a complete drill on the 0-day-old host, which was not fed on.

Of the 17 females whose first completed drill was on a 0-day-old host, only one subsequently completed a drill on the 3-day-old host as well; whereas of the 8 females whose first completed drill was on a 3-day-old host, 6 subsequently completed a drill on the 0-day-old host ($G = 13.04$, $P < 0.001$). Although only 7 females completed drills on both hosts, 23 attempted to drill both hosts. Eleven females made attempts on only 1 host; 5 made no attempts on either host.

Discussion

Consistent with the host quality model prediction, female *S. endius* produced a greater proportion of daughters from 0-day-old than from 3-day-old hosts when given a choice of host ages. They also did so when not given a choice. The sex ratio effect appears to result from mothers' oviposition behavior, not just from differential mortality of daughters and sons after oviposition: The greater failure of sons than daughters to exit older hosts is too small to explain the sex ratio effect, and the effect of host age on offspring survivorship during development was independent of maternal mating status. Two previous studies of *S. endius* found no differential mortality of the sexes, the same strain of *S. endius* parasitizing small and large hosts (Napoleon and King 1999) and a strain from Southwell, England, parasitizing young hosts (Donaldson and Walter 1984).

S. endius's sex ratio response to host age is consistent with the sex ratio response to host size in this same strain (Napoleon and King 1999). In contrast, host-size dependent sex ratios have not been found in *S. endius* from Southwell, England; from Indiana, USA; or from India (Donaldson and Walter 1984; King 1991; Napoleon and King, 1999). The host-age dependent sex ratios that have been found in other species of solitary parasitoid wasps also show an increased proportion of daughters from host ages that represent more food for the parasitoid, consistent with the host quality model prediction and the present study (Godfray 1994).

The host quality model assumes that poor host quality is more detrimental to daughters than sons. However, host age had no differential effect on daughters versus sons in terms of size, survivorship or duration of development. Thus, for *S. endius*'s sex ratio manipulation to be adaptive, the greater size or quicker development of offspring from 0-day-old hosts must provide a greater advantage to daughters than to sons. In addition, the advantage must be large enough to compensate for the disadvantage of sons being disproportionately trapped in 3-day-old hosts. Effects of body size on fitness have not been examined for *S. endius*. Ideally, fitness effects should be studied over multiple field seasons because effects vary with conditions even in the field (Heinz 1996; Kazmer and Luck 1995; West et al. 1996).

That the *S. endius* offspring from the younger hosts were larger as adults, developed more quickly, had greater survivorship and were better able to exit hosts may all be related. Larger offspring may have less difficulty chewing out of hosts. Or

perhaps the puparia of hosts that are parasitized when 0-day-old are less difficult to chew through. Less difficulty chewing out and perhaps in biting through the pupal integument (within the puparium) may speed development.

Previous experience with 0-day-old versus 3-day-old hosts had no apparent effects on a mother's subsequent fitness. Perhaps the greater difficulty drilling older hosts was offset by females completing fewer drills on them. Effects of host age on a mother's subsequent fitness have not been well-explored other than reports of increased risk of injury from older larval hosts as a result of their stronger defence behavior (e.g., Mattiacci and Dicke 1995). Whether older larval hosts are also more difficult to pierce or to adequately paralyze remains to be tested.

Although S. endius females differentially oviposited in 0-day-old versus 3-day-old hosts, there was no evidence of differential drilling attempts. Why is unclear. Old hosts are darker and have harder puparia than young hosts, and female S. cameroni distinguish between them even prior to antennation of the host (King 1998).

Acknowledgements I thank C. Geden for providing starter S. endius; R. Anderson, L. Baroody, J. Sanders and J. Smith for laboratory assistance; and M. Crowe, G. Heimpel, and R. King for comments on this manuscript. This research was supported by the Department of Biological Sciences at Northern Illinois University and complies with U.S. laws.

References

- Charnov EL (1982) The theory of sex allocation. Princeton University Press, Princeton
- Charnov EL, Los-den Hartogh RL, Jones WT, van den Assem J (1981) Sex ratio evolution in a variable environment. *Nature* 289:27--33
- Donaldson JS, Walter GH (1984) Sex ratios of Spalangia endius (Hymenoptera: Pteromalidae), in relation to current theory. *Ecol Entomol* 9:395--402
- Gerling D, Legner EF (1968) Developmental history and reproduction of Spalangia cameroni, parasite of synanthropic flies. *Ann Entomol Soc Am* 61:1436--1443
- Godfray HCJ (1994) Parasitoids. Princeton University Press, Princeton, New Jersey
- Heinz KM (1996) Host size selection and sex allocation behaviour among parasitoid trophic levels. *Ecol Entomol* 21:218--226
- Kazmer DJ, Luck RF (1995) Field tests of the size-fitness hypothesis in the egg parasitoid Trichogramma pretiosum. *Ecology* 76:412--425
- King BH (1990) Sex ratio manipulation by the parasitoid wasp Spalangia cameroni in response to host age: a test of the host-size model. *Evol Ecol* 4:149--156
- King BH (1991) No intersexual differences in host size and species usage in Spalangia endius (Hymenoptera: Pteromalidae). *Great Lakes Entomol* 24:17--20
- King BH (1994) How do female parasitoid wasps assess host size during sex-ratio manipulation? *Anim Behav* 48:511--518

- King BH (1998) Host age response in the parasitoid wasp Spalangia cameroni. J Insect Behav 11:103--117
- King BH, King RB (1994) Sex ratio manipulation in response to host size in the parasitoid wasp Spalangia cameroni: is it adaptive? Behav Ecol 5:448--454
- King BH, Lee HE (1994) Test of the adaptiveness of sex ratio manipulation in a parasitoid wasp. Behav Ecol Sociobiol 35:437--443
- Mattiacci L, Dicke M (1995) The parasitoid Cotesia glomerata (Hymenoptera: Braconidae) discriminates between first and fifth larval instars of its host Pieris brassicae, on the basis of contact cues from frass, silk, and herbivore-damaged leaf tissue. J Insect Behav 8:485--498
- Mousseau TA, Fox CW (1998) The adaptive significance of maternal effects. TREE 13:403--407
- Napoleon ME, King BH (1999) Offspring sex ratio response to host size in the parasitoid wasp Spalangia endius. Behav Ecol Sociobiol 46:325--332
- Rueda LM, Axtell RC (1985) Guide to common species of pupal parasites (Hymenoptera: Pteromalidae) of the house fly and other muscoid flies associated with poultry and livestock manure. Technical Bulletin 278. North Carolina Agricultural Research Service, North Carolina State University
- Sandlan KP (1979) Sex ratio regulation in Coccygomimus turionella Linnaeus (Hymenoptera: Ichneumonidae) and its ecological implications. Ecol Entomol 4:365--378
- West SA, Flanagan KE, Godfray HCJ (1996) The relationship between parasitoid size and fitness in the field, a study of Achrysocharoides zwoelferi (Hymenoptera: Eulophidae). J Anim Ecol 65:631--639

Table 1 Number of adult offspring when each mother received 10 0-day-old and 10 3-day-old hosts on each of two successive days.

	0-day-old hosts		3-day-old hosts	
	Mean \pm SE (Range)	n	Mean \pm SE (Range)	n
Day one	3.4 \pm 0.3 (0 - 8)	39	1.9 \pm 0.3 (0 - 8)	39
Day two	4.7 \pm 0.4 (0 - 9)	39	3.3 \pm 0.5 (0 - 9)	39

host age: $F_{1,38} = 18.54$, $P < 0.001$

day: $F_{1,38} = 14.76$, $P < 0.001$

host age by day: $F_{1,38} = 0.07$, $P = 0.80$

Conclusions are unaffected by excluding the 3 females that produced no offspring.

Table 2 Weight (mg) of female and male adult offspring from 0-day-old hosts and from 3-day-old hosts.

	0-day-old hosts		3-day-old hosts	
	Mean \pm SE (Range)	n	Mean \pm SE (Range)	n
Females	0.27 \pm 0.007 (0.23- 0.31)	14	0.26 \pm 0.009 (0.19- 0.31)	14
Males	0.17 \pm 0.005 (0.14 - 0.20)	14	0.15 \pm 0.004 (0.14 - 0.18)	14

host age: $F_{1,13} = 4.75$, $P = 0.048$

offspring sex: $F_{1,13} = 370.84$, $P < 0.001$

host age by offspring sex: $F_{1,13} = 0.63$, $P = 0.44$

Conclusions are unaffected by excluding an outlier, a "dwarf" female.

Table 3 Number of eggs from 10 hosts when each female, virgin or mated, received either 20 0-day-old or 20 3-day-old hosts.

	0-day-old hosts		3-day-old hosts	
	Mean \pm SE	n	Mean \pm SE	n
Virgin	5.5 \pm 0.6	33	4.3 \pm 0.6	33
Mated	7.3 \pm 0.5	33	5.2 \pm 0.6	33

host age: $F_{1,32} = 6.70$, $P = 0.014$

mating status: $F_{1,32} = 6.95$, $P = 0.013$

host age by mating status: $F_{1,32} = 1.10$, $P = 0.30$

Table 4 Duration of development (days) for offspring from 0-day-old hosts and from 3-day-old hosts.

	0-day-old hosts		3-day-old hosts	
	Mean \pm SE (Range)	n	Mean \pm SE (Range)	n
Female offspring	40 \pm 0.1 (38 - 45)	144	41 \pm 0.2 (38 - 47)	89
Male offspring	38 \pm 0.2 (36 - 47)	68	39 \pm 0.4 (35 - 44)	37

Host age: $F_{1,334} = 27.85$, $P < 0.001$

Wasp sex: $F_{1,334} = 193.36$, $P < 0.001$

Host age by wasp sex: $F_{1,334} = 0.09$, $P = 0.77$

The F values are for ranked data because assumptions of normality were not met; however, conclusions are unaffected by using untransformed data.