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Effects of host size experience on sex ratios in the parasitoid wasp Spalangia cameroni

BETHIA H. KING

Department of Biological Sciences, Northern Illinois University,
DeKalb, IL 60115, U.S.A.

Running headline: King: Host size experience effects on sex ratio

Abstract. The sex ratio response of a female Spalangia cameroni is affected both by the size of the host that she is parasitizing and by the size of hosts that she has previously encountered. When given small and large hosts simultaneously, Spalangia cameroni females oviposit a greater proportion of sons in the small hosts (King, 1988, Evolution, 42,1190-1198). This study examines how a female's offspring sex ratio is affected by her previous host size experience. First, van den Assem et al.'s life expectancy hypothesis was tested (Neth. J. Zool., 34,33-62). This hypothesis suggests that when females encounter only small hosts, the production of daughters will be inhibited initially and then, as no large hosts become available, production of daughters will increase. This hypothesis was not supported. The second hypothesis tested was that in their sex ratio responses, females will judge hosts as small or large relative to other hosts encountered. This hypothesis was supported under some conditions. Females judged hosts as small or large relative to previously encountered hosts when parasitizing large hosts and when the previous experience with hosts was immediately prior. When a female received only one host size, females that were given small hosts produced either a greater proportion of sons or the same sex ratio as females that were given large hosts.

Haplodiploidy provides parasitoid wasps with a physiological mechanism for maternal manipulation of sex: unfertilized eggs develop into sons, and fertilized eggs develop into daughters. Thus, mothers can potentially control offspring sex ratio (proportion of sons) in response to environmental conditions by adjusting the ratio of unfertilized eggs to fertilized eggs at oviposition. One of the environmental factors to which females of many species respond is host size (reviewed in King 1987, 1992). Here I examine the effects of host size experience on sex ratios in the parasitoid wasp Spalangia cameroni.

Spalangia cameroni parasitize pupae of certain fly species found in livestock manure (Rueda and Axtell 1985). S. cameroni is a solitary species, meaning that generally only one offspring develops on each host. Like most parasitoid wasp species that have been examined, when presented with both small and large hosts simultaneously, female S. cameroni produce a greater proportion of sons from small hosts than from large hosts (King 1988). In S. cameroni this pattern has been shown to be a result of maternal manipulation of offspring sex at oviposition, rather than a result of post-ovipositional differential mortality of the sexes (King 1988). Maternal control resulting in production of a greater proportion of sons in smaller hosts may be adaptive as described by host size models (Charnov 1979; Charnov et al. 1981) (also referred to more generally as host quality models).

Here, in the first experiment I test van den Assem et al.'s (1984) 'life expectancy hypothesis.' This hypothesis predicts that females that encounter only small hosts will increase the proportion of daughters they produce with time. The idea is that females that encounter only small hosts may initially postpone producing daughters in 'anticipation' of future encounters with large hosts; but as time passes and life expectancy decreases, the inhibition to lay daughters in small hosts may decrease (van den Assem et al. 1984). By similar reasoning, the hypothesis also predicts that when given only large hosts, females will exhibit an increase in the proportion of sons that they produce with time (van den Assem et al. 1984). This latter prediction is met in S. cameroni; however, this is not a critical test of the hypothesis because sperm depletion will lead to the same prediction. (Recall that with haplodiploidy, production of sons does not require sperm.)

In other experiments, I examine whether females judge host size in relative rather than absolute terms, as predicted by host size models (Charnov et al. 1981; Werren 1984). By judging host size in relative terms, I mean that a female's sex ratio response to a given host will depend on whether that host is smaller or larger than other hosts that she has encountered. Thus, she will treat small hosts as smaller (i.e. produce a greater proportion of sons in them) if her previous experience has been with large hosts versus small hosts. Similarly, she will treat large hosts as larger if her previous experience has been with small hosts versus large hosts. If she has encountered only a single host size, she might be expected to produce the same sex ratio regardless of absolute host size, because she has no other host size experience for comparison (King 1987). In contrast, if a female judges host size in absolute terms, her sex ratio response to a given host depends only on that host's absolute size and not on the sizes of other hosts that she has encountered. Here I examine (1) whether the sex ratio response to a given host depends on the host size distribution that a female has previously encountered, (2) whether more than 1 day of host experience is necessary for an effect on subsequent sex ratio responses to host size, (3) how long females are affected by

past host size experience, and (4) whether females given only small hosts produce the same sex ratios as females given only large hosts.

METHODS

General Methods

The *S. cameroni* used in this study were from a colony established in 1985 with wasps that emerged from *Musca domestica* and *Stomoxys calcitrans* pupae collected in Indiana (King 1991). I performed experiments at 23-28°C, continuous light, using *M. domestica* pupae that were less than 1-day-old as hosts (King 1988). I produced large and small hosts for the experiments by manipulating the volume of host eggs per volume of host media: 800 mm³ of eggs were reared in 1030 ml of larval medium to produce large hosts; 350 mm³ of eggs were reared in 130 ml of larval medium to produce small hosts (King 1988). I cannot be certain that there are not also quality differences between the two host size classes. However, the two size classes do provide different amounts of energy to developing wasps: both male and female *S. cameroni* are larger when they develop on larger hosts (King 1988).

Experimental females had developed on large hosts and were newly emerged (less than 1-day-old). Each female was paired with, and observed mating with, a newly emerged virgin male. I presented each female with hosts in a small plastic vial (40 mm high by 36 mm top diameter by 27 mm bottom diameter) with a drop of honey as food on the side of the vial and a drop of water in the bottom centre of the vial. The number and size of hosts that each female received is described under the individual experiments below. After offspring emerged, I counted them and determined their sex.

In the results presented here, I excluded females that copulated but produced only sons. However, such females accounted for only about 1% of all females and did not affect statistical significance of the results at $\alpha = 0.05$. When females in two different treatments received the same host size class, extra care was taken to ensure that pairs of females (one from each treatment) received hosts of the same size and age; thus, I compared such treatments by paired t-test (or by sign test if normality assumptions were not met).

Temporal Pattern of Response to Constant Host Size

I tested whether females encountering only small hosts increase the proportion of daughters that they produce as they get older, as the life expectancy hypothesis predicts. I gave each of 13 females 14 small hosts daily for 15 days, which is about half of their average reproductive longevity (personal observation). I examined the direction of the relationship between a female's offspring sex ratio and her age by Spearman rank correlation. Then, using a sign test, I tested whether more females exhibited negative relationships (i.e. a decrease in proportion of sons with increasing age) than positive relationships. I also used regression analysis to examine the relationship between mean offspring sex ratio of all females combined and maternal age.

Effect of Host Size Experience

To test the effect of 1 day of previous host size experience on sex ratio response

to large hosts, in each replicate, I gave one female 14 small hosts on day 1 and 14 large hosts on day 2 (treatment SL) and another female 14 large hosts on both days (treatment LL). To test the effect of 1 day of previous host size experience on sex ratio response to small hosts, in each replicate, I gave one female 14 large hosts on day 1 and 14 small hosts on day 2 (treatment LS) and another female 14 small hosts on both days (treatment SS). Thus, in both experiments, females in each treatment encountered the same host size on day 2 but encountered a different host size on day 1. In each experiment, I compared sex ratio and clutch size from day-2 hosts between treatments.

Because 1 day of previous host size experience had no effect on the subsequent sex ratio response to small hosts (see Results), I tested females after 3 days of experience with a particular host size. Thus, the treatments were SSSS versus LLLS. I compared the sex ratio and clutch size responses to small hosts on day 4 between treatments.

To address how long females are affected by previous host size experience, I repeated the SL versus LL experiment described above but allowed 1 hostless day between the first and second presentation of hosts. Thus, the treatments were SOL versus LOL. I compared the sex ratio and clutch size responses to large hosts on day 3 between treatments. I did not do a corresponding SOS versus LOS experiment because there had been no effect of prior host size experience on sex ratio response to small hosts in the other experiments (see Results).

Relative versus Absolute Assessment of Host Size

In the above experiments, in addition to comparing the sex ratio response on the final day when females in both treatments were given the same host size, I also compared the sex ratios of females when they were given different host sizes (i.e. the first one to three days). These comparisons were by t-tests, or by Mann-Whitney U tests when assumptions of normality were not met, or by MANOVA's (multivariate analyses of variance) when multiple days of response were examined. In the MANOVA's, sex ratio or clutch size on day 1, day 2, and day 3 were response variables, and host size (small and large) was a between-subjects factor (Norusis 1988). MANOVA's were followed by univariate F tests to determine which days contributed to the differences found by MANOVA.

RESULTS

Temporal Pattern of Response to Constant Host Size

Females that encountered only small hosts did not increase the proportion of daughters that they produced as they got older. The number of mothers exhibiting a positive Spearman rank correlation between their proportion of daughters and their maternal age was not significantly greater than the number exhibiting a negative correlation (seven versus six, sign test $P = 1.00$). Across all mothers, there was no significant relationship between mean sex ratio and maternal age (Fig. 1; Spearman rank correlation = -0.004, $P = 0.95$). Closer analysis suggested that the proportion of daughters increased from day 1 to two (Fig. 1; $t = 3.44$, $df = 23$, $P = 0.003$). However, such an increase also occurred when females were given only large hosts (Table I), and the amount of increase did not differ between mothers given only small hosts and

mothers given only large hosts ($t = 0.75$, $df = 61$, $P = 0.45$).

Effect of Host Size Experience

I found some evidence that previous host size experience affected sex ratio. Sex ratio response to large hosts was significantly more female-biased for mothers that had been previously exposed to small hosts for 1 day than for mothers that had been previously exposed to large hosts for 1 day (Table II). Clutch size response to large hosts was also affected by previous host size experience, with fewer offspring being produced by females that had been exposed to small hosts previously than by females that had been exposed to large hosts (Table II). The lower proportion of sons and smaller clutch sizes produced by females exposed to small hosts the previous day do not appear to be related: in both the SL and the LL treatments, the relationship between proportion of sons and clutch size was non-significant ($r = -0.001$, $df = 23$, $P = 0.99$; $r = -0.25$, $df = 23$, $P = 0.22$). The effect of previous host size experience on sex ratio and clutch size responses to large hosts was not found when females were given no hosts for a day before receiving the large hosts (Table II).

In contrast to the effect of host size experience on sex ratio and clutch size responses to large hosts, there were no significant effects of previous experience on the responses to small hosts (Table II). Even when females were exposed to a given host size for 3 days, there were no significant effects of host size experience on responses to small hosts (Table II).

Relative versus Absolute Assessment of Host Size

When females received hosts of only a single size category, the sex ratio produced by females given large hosts was either not significantly different from the sex ratio of females given small hosts or was more female-biased. In two experiments there were no significant effects of host size on sex ratio (Tables I and III); in another experiment, there was a greater proportion of daughters from mothers that received large hosts than from mothers that received small hosts (Table III). The effect on clutch size of receiving different host sizes varied among and within experiments. In two experiments, clutch size did not differ between mothers receiving small versus large hosts (Table III). In another experiment, there was a significant overall effect of host size on clutch size; on day 2 more offspring were produced from large hosts, and on day 3 more offspring were produced from small hosts (Table I).

DISCUSSION

Temporal Pattern of Response to Constant Host Size

Spalangia cameroni females did not exhibit a steady increase in the proportion of daughters they produced over their first 15 days (Fig. 1), and the increase exhibited from day 1 to 2 was no greater than when females had encountered only large hosts (Table I). Thus, my results do not support the life expectancy hypothesis. Sperm depletion may prevent females from being able to increase their proportion of daughters (Hurlbutt 1987). Similar to *S. cameroni*, *Anisopteromalus calandrae* females increase the proportion of daughters they produce from the first to the third day, not

only when they are given only small hosts, but also when given only large hosts (van den Assem et al. 1984). A greater proportion of sons on the first day than on subsequent days may result from sperm requiring time to reach the spermatheca and thus not being fully available on the first day (Wilkes 1965).

Effect of Host Size Experience

A female's sex ratio and clutch size response to large hosts was affected by whether she had encountered small or large hosts the previous day. However, the lower proportion of sons and smaller clutch size produced by females exposed to small hosts the previous day (Table II) do not appear to be related: within treatments there was no evidence of a positive relationship between proportion of sons and clutch size.

One explanation for why females produced a greater proportion of daughters from large hosts when they had encountered small rather than large hosts the previous day is that females may remember previously encountered hosts, and large hosts may seem larger when past experience was with small hosts. If females remember previous host size experience, it is not for more than about 1 day (Table II). If females remember previous host size experience when ovipositing on large hosts, it is not clear why they do not remember when ovipositing on small hosts (Table II).

Females exposed to small hosts the previous day produced fewer offspring in large hosts than females previously exposed to large hosts (Table II), but the reason for this is not clear. One hypothesis is that it may result from nutritional effects on the female. In addition to laying eggs in hosts, females also feed on fluids exuding from the host after they drill into it for oviposition. Large hosts may provide more food than small hosts. Host feeding is necessary for continued egg production (Gerling & Legner 1968). However, if this nutritional explanation is correct, it is not clear why previous exposure to large hosts versus small hosts did not affect clutch size response to small hosts, even when females were given 3 days of prior host size experience (Table II). In addition, the effect of previous host size experience on clutch size response to large hosts was absent when females were given no hosts for a day before receiving the large hosts (Table II); yet it seems unlikely that this loss of clutch size effect was due to egg resorption as females are not reported to undergo resorption until after 10 days without hosts (Gerling & Legner 1968).

The effect of previous host size experience on sex ratio behavior has been examined in only a few species of parasitoid wasps. An effect of previous host size experience on sex ratio has been found in A. calandrae and Ephedrus californicus (van den Assem et al. 1984; Cloutier et al. 1991).

Relative versus Absolute Assessment of Host Size Previous experiments had suggested that female S. cameroni judge the size of a host relative to the size of previously encountered hosts, as predicted by host size models (Charnov et al. 1981). The proportion of sons was lower from large hosts when both large and small hosts were presented simultaneously than when large hosts were presented alone (King 1991). Similarly, the proportion of sons was higher from small hosts when both small and large hosts were presented simultaneously than when small hosts were presented alone. Also, when given only hosts of a single age category (host age is related to host weight), sex ratio was independent of host age (King 1990).

In contrast, in the experiments reported here, among females that were each given only one size of hosts, females that were given small hosts produced either the

same sex ratio or a greater proportion of sons than females that were given large hosts. Thus, a female's sex ratio response to host size does not seem to rely strictly on relative size cues, rather females seem to use a combination of relative and absolute cues in measuring host size. A similar situation may be seen with the parasitoid wasp A. calandrae. Females in this species judge host size using both relative and absolute cues: the sex ratio from a given host size varies according to what host sizes have been previously encountered; yet when each female encounters only one host size, sex ratio among females varies with host size (van den Assem et al. 1984). Data available to date suggest that among other solitary species, Pimpla instigator, Lariophagus distinguendus, Diglyphus begini, and Antrocephalus pandens females judge host size in relative terms (Chewyreu 1913 in Clausen 1939; van den Assem 1971; Heinz & Parrella 1989, 1990; Brault 1991); and Heterospilus prosopidis females judge in absolute terms (Charnov et al. 1981; Jones 1982). However, the results with A. calandrae and S. cameroni suggest that how females of these other species judge host size may prove to be more complex.

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Table I. Sex ratio (proportion of sons) and clutch size on days 1-3 produced by females given small hosts for their first 3 days versus by females given large hosts for their first 3 days.

| | Day 1 | Day 2 | Day 3 |
|--------------------------|----------------------|----------------------|----------------------|
| | $\bar{X} \pm SE (n)$ | $\bar{X} \pm SE (n)$ | $\bar{X} \pm SE (n)$ |
| Sex ratio [*] | | | |
| Small hosts | 0.19 ± 0.027 (31) | 0.14 ± 0.017 (31) | 0.13 ± 0.019 (31) |
| Large hosts | 0.18 ± 0.016 (31) | 0.10 ± 0.016 (31) | 0.10 ± 0.014 (31) |
| Clutch size [†] | | | |
| Small hosts | 9.7 ± 0.57 (32) | 8.2 ± 0.40 (32) | 9.9 ± 0.39 (32) |
| Large hosts | 9.6 ± 0.51 (32) | 9.4 ± 0.38 (32) | 8.7 ± 0.42 (32) |

^{*}MANOVA approximate $F_{3,58} = 1.43$, $P = 0.24$.

[†]MANOVA approximate $F_{3,60} = 3.11$, $P = 0.03$;

day 1: $F_{1,62} = 0.03$, $P = 0.87$;

day 2: $F_{1,62} = 5.09$, $P = 0.03$;

day 3: $F_{1,62} = 4.57$, $P = 0.04$.

Table II. Sex ratio (proportion of sons) and clutch size produced by females on the last day of each treatment.

| Treatment | Sex ratio | Clutch size |
|-----------|----------------------------------|----------------------------------|
| | $\bar{X} \pm SE (\underline{n})$ | $\bar{X} \pm SE (\underline{n})$ |
| SL | 0.13 \pm 0.020 (25) | 8.0 \pm 0.48 (25) |
| LL | 0.19 \pm 0.014 (25) | 9.4 \pm 0.42 (25) |
| | $\underline{t} = 2.56$ | $\underline{t} = 2.39$ |
| | $\underline{P} = 0.009$ | $\underline{P} = 0.03$ |
| LS | 0.19 \pm 0.025 (20) | 8.4 \pm 0.59 (22) |
| SS | 0.24 \pm 0.048 (20) | 7.9 \pm 0.63 (22) |
| | $\underline{t} = 1.33$ | $\underline{t} = 0.63$ |
| | $\underline{P} = 0.20$ | $\underline{P} = 0.54$ |
| SOL | 0.15 \pm 0.019 (33) | 11.1 \pm 0.51 (34) |
| LOL | 0.17 \pm 0.014 (33) | 10.8 \pm 0.45 (34) |
| | $\underline{t} = 0.95$ | sign test |
| | $\underline{P} = 0.18$ | $\underline{P} = 0.25$ |
| SSSS | 0.13 \pm 0.018 (32) | 10.5 \pm 0.36 (32) |
| LLLL | 0.16 \pm 0.019 (32) | 9.8 \pm 0.42 (32) |
| | $\underline{t} = 1.05$ | $\underline{t} = 1.45$ |
| | $\underline{P} = 0.15$ | $\underline{P} = 0.16$ |

SL: small hosts on day 1, large hosts on day 2; LL: large hosts on day 1 and day 2; LS: large hosts on day 1, small hosts on day 2; SS: small hosts on day 1 and on day 2; SOL: small hosts on day 1, no hosts on day 2, large hosts on day 3; LOL: large hosts on day 1, no hosts on day 2, large hosts on day 3; SSSS: small hosts on days 1-4; LLLL: large hosts on days 1-3, small hosts on day 4

*Based on arcsine square root-transformed data

Table III. Sex ratio (proportion of sons) and clutch size on day 1 from females given small hosts versus from females given large hosts on day 1.

| Treatment | Sex ratio | Clutch size |
|--|----------------------------------|----------------------------------|
| | $\bar{X} \pm SE (\underline{n})$ | $\bar{X} \pm SE (\underline{n})$ |
| SL versus LL and SS versus LS experiments: | | |
| Small hosts | 0.23 \pm 0.019 (48) | 10.0 \pm 0.30 (48) |
| Large hosts | 0.24 \pm 0.025 (46) | 9.7 \pm 0.38 (46) |
| | $\underline{U} = 1093.5$ | $\underline{t} = 0.63$ |
| | $\underline{P} = 0.94$ | $\underline{P} = 0.53$ |
| SOL versus LOL experiment: | | |
| Small hosts | 0.29 \pm 0.021 (31) | 10.1 \pm 0.47 (31) |
| Large hosts | 0.24 \pm 0.019 (34) | 10.0 \pm 0.47 (34) |
| | $\underline{t} = 1.80$ | $\underline{t} = 0.15$ |
| | $\underline{P} = 0.04$ | $\underline{P} = 0.89$ |

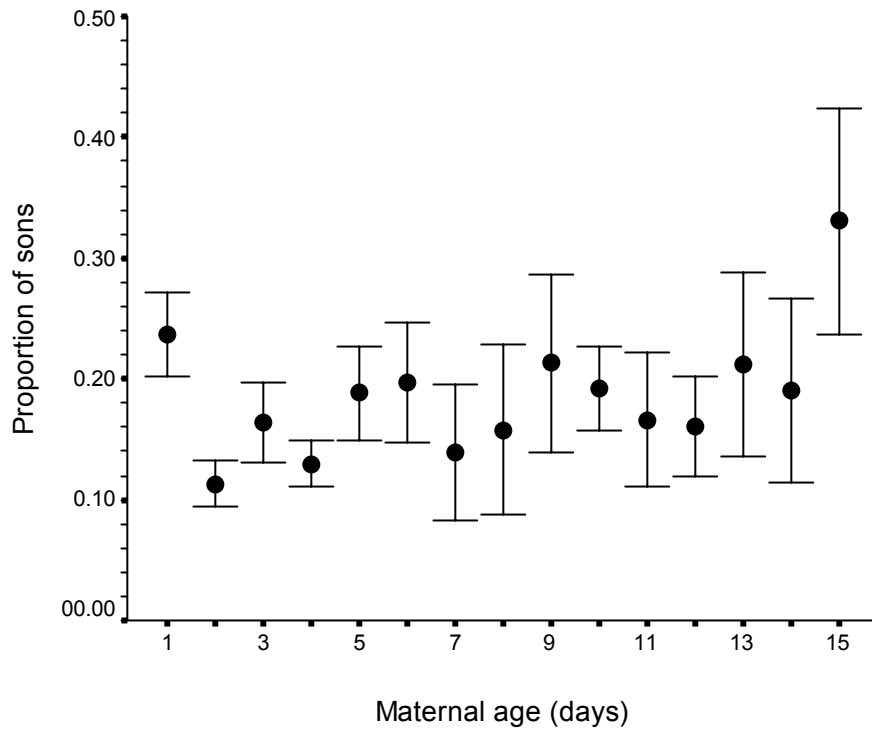


Figure 1. Mean \pm SE proportion of sons produced by females given small hosts daily for 15 days.