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Sex ratio manipulation by the parasitoid wasp *Spalangia cameroni* in response to host age: a test of the host size model

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Summary

A sex ratio response to host resources as measured by external host dimensions has been demonstrated in many parasitoid wasps, including *Spalangia cameroni*. The responses generally are in the direction predicted by sex ratio theory, specifically the host size models. Here I show that female *S. cameroni* also respond to differences in resource availability not associated with changes in external host dimensions, and this response is in the direction predicted by host size models. When given old and young hosts simultaneously, female *S. cameroni* oviposit a greater proportion of sons in old than in young host pupae, at least for 0-day old versus 3-day old hosts. Old hosts weigh less than young hosts but are not significantly different in external width. Thus it appears that the offspring sex ratio response may result from mothers detecting physical or chemical changes within the host which are associated with host age. No evidence is found that the manipulation in response to host age has been selected for via an effect of host age on wasp size: there was no significant effect of host age on either male or female wasp size. A second prediction of the host size models is also supported by this study: when each female is presented with only a single host age, rather than two host ages simultaneously, host age has no effect on offspring sex ratio.

Introduction

One variable which may affect which sex ratio a female should produce is the amount of resources which will be available to her offspring (Trivers and Willard, 1973). This idea has been developed into formal evolutionary models, referred to as host-size models, for solitary parasitoid wasps (Charnov, 1979; Charnov et al., 1981). Parasitoid wasps provision their offspring with food by ovipositing in or on a host (typically an insect). In solitary species by definition only one offspring completes development on each host. The host size models suggest that females of solitary species should manipulate the sex of their offspring in response to characteristics of the hosts on which they oviposit. The models consider host size in particular,

but are generalizable to any host characteristic which has a differential effect on male and female reproductive success (Charnov et al., 1981; Charnov, 1982). Here I examine whether the solitary parasitoid wasp *Spalangia cameroni* manipulates offspring sex ratio in response to host age in a manner consistent with the host size models.

The host size models predict that for solitary species of parasitoids, mothers should oviposit a greater proportion of daughters in large than in small hosts when they encounter both host sizes. The critical assumption is that developing on a large host confers more to the ultimate reproductive success of females than of males. Charnov et al. (1981) suggested that host size may have a greater effect on female than on male reproductive success if host size is positively correlated with wasp size and if wasp size increases the reproductive success of females more than the reproductive success of males. Alternatively, host size may have a differential effect on reproductive success of females versus males through an effect on development time (Hurlbutt, 1987; King, 1988). For example, the development time of males and females may be differentially affected by host size (King, 1988). Decreased development time might increase a wasp's reproductive success by decreasing generation time and/or by decreasing the period of exposure to predation and parasitism while the wasp is developing.

For most species of parasitoid wasps examined, including *S. cameroni*, there is a negative relationship between proportion of sons and host size as predicted by the models (King, 1987, 1988, 1989a). The relationship between sex ratio and host age has not previously been examined for *S. cameroni*. For parasitoids of eggs or pupae, such as *S. cameroni*, host size models should predict a positive relationship between host age and proportion male parasitoids because eggs and pupae are expected to provide fewer resources with increasing host age. Neither eggs or pupae feed, but they do metabolize and some resources may be converted into substances which cannot be assimilated by developing parasitoids [e.g., pupal hosts of *S. cameroni* (Gerling and Legner, 1968)]. In contrast, host size models predict a negative relationship between parasitoid sex ratio and host age for parasitoids of larvae. This is because size and age are positively correlated for larvae. The effect of host age on wasp offspring sex ratio has been examined for more than twenty larval parasitoids (reviewed in King, 1987, 1989a). The effect of host age on wasp sex ratio for egg and pupal parasitoids has received considerably less attention (see Discussion), and this is the first study designed to test the effect of host age on a pupal parasitoid's sex ratio as a test of the host size model.

A significant relationship between a host character and wasp sex ratio is not complete evidence that females are manipulating offspring sex ratio in response to that character. An alternative explanation is differential mortality of the sexes among the developing offspring, e.g., through starvation. Here I test for differential mortality of the sexes by comparing the adult offspring production of virgin versus mated females (Sandlan, 1979; King, 1988). Because of the haplodiploid sex determination, virgin females produce only sons, whereas mated females produce both sons and daughters. If there is differential mortality of the sexes, production of adult offspring will differ between the two types of females. For example, if there is greater mortality of daughters than of sons during development, mated mothers will produce fewer adult offspring than will virgin mothers. This test for differential mortality assumes that the two types of females parasitize the same number of hosts initially, so that any difference in production of adult offspring results from offspring mortality, rather than from differences in parasitization rate between virgin and mated mothers. Earlier work with *S. cameroni* showed no difference in offspring production between virgin and mated mothers, suggesting no differential mortality of the sexes (King, 1988). Here I provide a more thorough test for differential mortality of the

sexes by comparing estimates of offspring survivorship between virgin and mated mothers.

Testing the host size models also requires examining the assumption of the models that the host character has a greater effect on the reproductive success of females than of males. Here I examine the effect of host age on size and development time of male and female *S. cameroni* as routes by which host age may differentially affect female versus male reproductive success.

An additional prediction of the host size models is that when there is temporal variation in host size distributions [as there is for *S. cameroni* (King, MS1)], mothers should judge hosts in relative terms, e.g., a mother should view a host as large only if she encounters other smaller hosts as well (Charnov et al., 1981; Charnov, 1982). *S. cameroni* does appear to judge host size in relative terms (King, MS1) as do some other parasitoid wasps (Chewyreu, 1913 in Clausen, 1939; van den Assem, 1971; Charnov et al., 1981). Here I examine whether *S. cameroni* also judges host age in relative terms.

Materials and methods

General methods

All experiments were conducted with laboratory populations of *Spalangia cameroni* and one of its natural hosts, pupae of the house fly *Musca domestica*. The *S. cameroni* colony was originated from wasps that emerged from *M. domestica* and *Stomoxys calcitrans* (L.) pupae collected in 1985 (King, MS1). Methods for maintenance of the laboratory populations are presented elsewhere (King, 1988). Experiments were performed at 22-23 C, 24L.

Wasps used in experiments were newly emerged and had not been exposed to other wasps except when mated. Mating was with a newly emerged virgin male in a 1 oz vial and was observed. A spot of honey was put on the vial wall as food for the female.

Host pupae used in experiments were of consistent size (produced following the procedure in King (1988) for large hosts). Host age classes used in experiments were young hosts (0-day-old) and old hosts (1- or 3-day-old, depending on the experiment). When presented to the wasps, 0-day-old hosts were less than 24 hours old (timed from the initiation of pupal tanning). Hosts were presented to females in 1 oz vials. In all experiments, there was a ridge of used host media across the center bottom of the vial, with seven hosts on either side. The ridge was used so that when young and old hosts were presented simultaneously, they could be kept separate and thus distinguishable. Mothers moved readily across the ridge of media.

To test the premise that older fly pupae provide fewer resources than young pupae, the oven-dry weights of thirty-two 0-day-old and thirty 3-day-old pupae were compared. The widths of twenty fly pupae which had not been oven-dried were measured at 0-, 1-, and 3-days-old to the nearest 0.03 mm to determine if host external dimensions changed with age.

For each experiment, offspring sex ratios or viabilities were computed across days for each mother, i.e., mother was the sampling unit. Replicates of treatments were matched temporally, and so statistical comparisons were by paired t-tests when assumptions of normality and equal variances were met and by Mann-Whitney U tests when these assumptions were not met.

Effect of host age on offspring sex ratio, size, and development time

To determine whether mothers manipulate sex ratio in response to host age, mated mothers were each given seven young and seven old hosts simultaneously for each of two days, either seven 0-day-old and seven 1-day old hosts or seven 0-day-old and seven 3-day-old hosts. All

potentially parasitized hosts were isolated in gelatin capsules, and development time of each wasp offspring was determined to the nearest day. Oven-dry weights of wasps were measured with a Mettler AE163 balance.

In looking at the effects of host age on offspring size and development time, I report comparisons of means; and when the outcome differed from the comparison of means, I also report sign test results. Each test has its own bias: calculations of means may be biased by maternal effects if there are differences among mothers in clutch size; sign tests exclude mothers which do not produce offspring in both host ages.

To determine the effect of host age on offspring sex ratio when mothers were given just one host age, mated mothers were given either fourteen 0-day-old hosts or fourteen 3-day-old hosts for each of two days, and the sex ratio of the resulting offspring was determined.

Differential Mortality of the Sexes

To test for differential mortality of the sexes, in addition to the mated females given fourteen 3-day-old hosts daily for two days in the above experiment, virgin females also were each presented with fourteen 3-day-old hosts for each of two days. For both the mated and the virgin mothers, in half of the replicates the wasp offspring were allowed to develop into adults before being sexed and counted. In the other half of the replicates, half of the hosts (seven from day one and seven from day two) were dissected and wasp eggs counted. Viability (number of adult offspring per egg laid) was compared between virgin and mated mothers.

Results

Effect of Host Age on Host Weight and Width

As expected, fly pupae lost weight with age (0-day versus 3-day-old pupae: mean + s.e.=6.45 mg + 0.100 versus 6.05 mg + 0.087, $t = 3.01$, $P = 0.002$; ranges 5.50-7.90 mg versus 5.40-7.00 mg). Width of fly pupae did not decrease with age (0-day versus 1-day old pupae: $t = 0.27$, $P = 0.79$; 0-day versus 3-day-old pupae: $t = 0.77$, $P = 0.23$).

Offspring Sex Ratio Patterns

When mothers were given seven 0-day-old and seven 1-day old hosts simultaneously, there was no significant difference in offspring sex ratio produced between the two host ages (Table 1). However, the means are in the expected direction. When mothers were given seven 0-day-old and seven 3-day-old hosts simultaneously, a significantly greater proportion of sons emerged from 3-day-old than from 0-day-old hosts (Table 1). This appears to be due to an increased number of sons ($P = 0.06$), not a decreased number of daughters ($P = 0.24$) from old hosts. When females were given just one host age, sex ratios were not significantly different between host ages (Table 1).

Differential Mortality of the Sexes

There was no evidence of differential mortality of the sexes. Virgin and mated mothers showed no significant difference in production of adult offspring per egg oviposited (mean + s.e.=0.88 + 0.097 versus 1.03 + 0.116; $t = 0.86$, $df = 16$, $P = 0.41$).

Effect of Host Age on Offspring Size and Development Time

Host age had no significant effect on weight of either male or female offspring when mothers

were given 0-day-old and 3-day-old hosts simultaneously (Table 2).

When mothers were given 0-day-old and 1-day old hosts simultaneously there was no effect of host age on development time of sons (Table 3). When mothers were given seven 0-day-old and seven 3-day-old hosts simultaneously, the effect of host age on male development time was non-significant by a comparison of means (Table 3). However, for 10 of 11 mothers which produced sons from both host ages, average son development time was less on 3-day-old hosts (sign test, $P = 0.01$).

The effect of host age on female development time is difficult to interpret. There was some indication of an increase in development time on older hosts for 0-day versus 1-day-old hosts, but not for 0-day versus 3-day-old hosts. A comparison of means showed development time of daughters was significantly less on 0-day-old than on 1-day old hosts (Table 3); however, a sign test was not significant: average development time of daughters was less on 0-day-old than on 1-day old hosts for only eleven of seventeen mothers (sign test, $P = 0.33$). There was no significant effect of host age on female development time with 0-day versus 3-day-old hosts by either a comparison of means or a sign test (Table 3).

Discussion

Results of this study provide some support for some basic predictions of the host size models. Old hosts weigh less in dry weight than do young hosts, and so old hosts are expected to provide fewer resources to a parasitoid. The experiments reported here show that, as predicted, *S. cameroni* females produce a greater proportion of sons in old than in young hosts when presented with both host ages simultaneously.

This pattern does not appear to be a result of differential mortality of daughters in old hosts. In most parasitoid wasps, including *S. cameroni*, females are larger than males (Hurlbutt 1987; King, 1988; Table 2). Thus, the lower resource availability on old hosts might be expected to increase a female's risks of starvation more than a male's. However, offspring survivorship from egg to adult on old hosts did not differ significantly between virgin and mated mothers, and was not even in the direction expected if there was starvation of daughters. This and the increase in absolute number of sons from old hosts versus young hosts indicates that the greater proportion of sons from old than from young hosts was a result of maternal manipulation.

That *S. cameroni* manipulate offspring sex ratio in response to host age in the manner predicted by the host size model is consistent with earlier findings that females manipulate offspring sex ratio in response to host size in the manner predicted by the host size model (King, 1988). In the earlier study (King, 1988), hosts were similar in age but differed in external dimensions. That the increase in proportion sons from 0-day-old to 3-day-old hosts was only eleven percent probably results from the large overlap in the amount of resources that the two host ages provide. Host age may be less accurate an indicator of host resources than the cue that females are actually using in their sex ratio response.

Another sex ratio pattern predicted by the host size models is that when a female encounters only a single host size, sex ratio should not vary according to the host size presented (Charnov, 1979; Werren, 1984). This prediction is related to the more general prediction that females should judge host size in a relative rather than an absolute manner (Charnov et al., 1981). *S. cameroni* has been shown to judge host size in relative terms when manipulating offspring sex ratio in response to host size (King, MS1). Similarly, the current study shows that when given just 0-day-old or just 3-day-old hosts, the sex ratio produced by a female did not vary according

to the host age (Table 1).

Regardless of host sizes encountered, females consistently produced female-biased overall sex ratios (Table 1). A male-biased overall sex ratio is a prediction of a pure host size model, i.e., when local mate competition and inbreeding are absent (Charnov et al., 1981; Werren, 1984). The female-biased overall sex ratio of *S. cameroni* can be explained by the presence of local mate competition in this species (King, 1989b).

This study provides further evidence that *S. cameroni* manipulates offspring sex ratio in ways predicted by the host size models. However, it is still not clear whether *S. cameroni* fits the assumptions of the host size models and thus that the sex ratio manipulation in response to host characteristics is adaptive. Specifically, the results do not provide much support for the host size models' assumption of a greater effect of host size/age on female than on male reproductive success. There was no discernable effect of host age on wasp size for either male or female wasps, and the effect of host age on development time was very small, when present.

The weak suggestion from the experiments reported here that development time may be important is at least consistent with the observed effect of host age on sex ratio: there was some indication that sons develop more quickly on older hosts, and mothers preferentially oviposit sons on older hosts. Earlier studies on the effect of host size on *S. cameroni*'s size and development time (King 1988) showed 1) a positive effect on both male and female wasp size, but no effect of host size on either male or female reproductive success through an effect on wasp size, and 2) a decrease in female development time in larger hosts. In both the present study with host age and the earlier one with host size, the effect on development time has been slight (less than one day). Further tests on the effects of host age and size on aspects of male and female wasp reproductive success are needed.

The effect of host age on offspring sex ratio has been examined in a small number of other solitary parasitoids which parasitize the pupal or egg stage of hosts. When mothers are given different host ages simultaneously, the pupal parasitoids *Coccygomimus dispar* and *Muscidifurax raptor* show no directional effects of host age on offspring sex ratio (Fuester et al., 1989; Podoler and Mendel, 1979); the egg parasitoid *Edovum puttleri* shows a positive relationship in one experiment and no directional relationship in another experiment (experiments 1 and 2 respectively of Table 2 in Lashomb et al., 1987). The role of differential mortality has not been determined for *E. puttleri*. Of these three species, the effect of host age on wasp development time has been examined in *M. raptor*: there is a similar decrease in development time on older hosts for both males and females (Podoler and Mendel, 1979). The effect of host age on wasp size has been examined for *Edovum puttleri* (Ruberson et al., 1987): males and females both were smaller when they had been oviposited on older hosts.

The effect of host age on offspring sex ratio when each mother is given only a single host age also varies among solitary parasitoid wasps. Like *S. cameroni*, the pupal parasitoid *Muscidifurax zaraptor* and the egg-larval parasitoid *Ascogaster reticulatus* show no significant relationship (Mandeville et al., 1988; Kainoh, 1988). (Whether or not these two species exhibit any effect of host age on parasitoid sex ratio when a single mother is given multiple host ages simultaneously has not been determined.) The egg parasitoid *E. puttleri* shows a positive relationship in one study (Lashomb et al., 1987) and in another study, a positive relationship for one biotype and no relationship for another biotype (Ruberson et al., 1987).

The proximate mechanisms of sex ratio manipulation in response to host characteristics are largely unknown. There is some evidence that external host parameters are important in oviposition decisions in some species of parasitoid wasps. The pupal parasitoid wasp

Coccygomimus turionellae manipulates offspring sex ratio in response to cocoon size rather than size of the host within (Aubert, 1961; Sandlan, 1979). In deciding what clutch size to oviposit in different sized hosts, *Trichogramma minutum*, a parasitoid wasp which parasitizes the egg stage of certain insects, appears to judge host size using external parameters--measuring how long it takes to walk across the host surface (Schmidt and Smith, 1987). For *S. cameroni*, there was no detectable decrease in external host width with increasing host age, though there was a clear decrease in host weight. These results suggest that *S. cameroni*'s manipulation of offspring sex ratio in response to host age may be a response to changes within the host puparium associated with host age, rather than a response to external host dimensions. Further experiments will be needed to determine more specifically what cues females are using in their sex ratio response. For example, are mothers responding to the smaller size of the fly pupae within the puparia or to physical or chemical characteristics of the fly pupa associated with its age? Other indications that female parasitoid wasps manipulate offspring sex ratio in response to host quality changes not associated with changes in external host dimensions come from work with the pupal parasitoid *Pachycrepoideus vindemiae* (van Alphen and Thussissen, 1983). This species responds to host quality changes associated with the host pupa having been previously parasitized by another species of parasitoid.

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Table 1. Mean + s.e. proportion sons when mothers were presented with either seven young and seven old hosts or with fourteen hosts all of the same age.

7 0-day hosts	0.19 + 0.017	t = 0.48, df = 16, P = 0.32
7 1-day hosts	0.21 + 0.027	
combined	0.20 + 0.008	
7 0-day hosts	0.20 + 0.050	t = 2.02, df = 16, P = 0.03
7 3-day hosts	0.31 + 0.048	
combined	0.26 + 0.040	
14 0-day hosts	0.22 + 0.018	t = 0.71, df = 15, P = 0.49
14 3-day hosts	0.20 + 0.021	

Table 2. Mean + s.e. wasp oven dry weight (mg) from young and old hosts.

Host Age	Males		Females	
	Mean + s.e.	n	Mean + s.e.	n
0-day	0.21 + 0.006	18	0.27 + 0.003	55
3-day	0.22 + 0.006	27	0.27 + 0.003	49
	t = 0.81, P = 0.42		t = 0.57, P = 0.57	

Table 3. Mean + s.e. wasp development time (in days) from young and old hosts.

Host Age	Males		Females	
	Mean + s.e.	n	Mean + s.e.	n
0 day	36.14 + 0.271	28	38.04 + 0.121	127
1 day	35.86 + 0.163	29	38.30 + 0.133	103
	Mann-Whitney U = 359.0, P = 0.44		Mann-Whitney U = 5391.0, P = 0.02*	
0 day	36.06 + 0.340	34	38.21 + 0.104	122
3 day	35.52 + 0.211	48	38.03 + 0.101	115
	Mann-Whitney U = 700.0, P = .26*		t = 1.23, P = 0.22	

*but see text for different result of sign test