Sex Ratio and Host Size in a Parasitoid Wasp

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Summary. Parasitoid wasps often lay male eggs in small hosts and female eggs in larger hosts. The selective advantage of this strategy can be explained by assuming wasp fitness increases with host size and that this fitness increase is greater in females than in males. I conducted experiments to test a model based on this explanation and found the results generally supported the model with one exception; unlike what the model assumed, these wasps were unable to adjust their offspring sex ratios in each generation to different host size distributions. This finding suggests an alternate view as to how selection might operate in the evolution of parasitoid sex ratios.

Introduction

Fisher (1930) first explained how natural selection should determine the equilibrium sex ratio of populations. He pointed out that since each sex contributes half the total genetic material to future generations, the reproductive investments in each sex should be equal. However, in some organisms, individuals of one sex may be more expensive to produce than of the other, and so the parental investment per individual is not equal in the two sexes. One such case is that of parasitic Hymenoptera attacking variable size hosts. Charnov (1979) has devised a model to explain how parents should allocate their resources to males vs females in this situation. Here I describe experiments that test the predictions and assumptions of that model.

These experiments used populations of one host species and one parasitoid species derived from laboratory stocks. The host species was the Azuki bean weevil, *Callosobruchus chinensis*, which lays its eggs on the surface of Azuki beans, *Phaseolus radiatus aurea*. After five days (at $T=30°$ C) the eggs hatch,

the larvae bore into the bean, and emerge as adults sixteen days later. The parasitoid wasp was *Heterospilis prosopidis* Vier., a solitary endoparasitic braconid that is not a natural parasitoid of the Azuki bean weevil. Female wasps locate weevil larvae inside beans, then insert their ovipositors, and lay one egg per host individual. Adult wasps emerge from the beans 11 to 13 days later (at $T=30°$ C), males tending to emerge before females. Female wasps encounter host larvae in various developmental stages, but only larvae aged 11 to 19 days (measured from the time of egg laying) provide suitable hosts.

The fitnesses of wasps from different size hosts within the 11 to 19 day old range may not be equal. If wasp fitness is related to body size, older and larger hosts provide more food for a developing parasite and produce larger more fit wasps. Assuming this effect on fitness is more pronounced in females than in males, since females must produce larger gametes, a female wasp can maximize her genetic contribution to later generations by laying males in small hosts and females in large hosts. Since the wasps are haplodiploid, females can control the sex of individual offspring by controlling fertilization (Flanders 1956).

Charnov's model predicts how wasps should distribute their male and female progeny over the available hosts in order to maximize offspring fitness. According to his model, the relative rather than absolute sizes of hosts present determine the sex ratio laid in a particular host size. Female wasps estimate host size distributions and adjust the sex ratios to maximize the collective fitness of their offspring. For example, if a host population consists of 50% small hosts and 50% medium-size hosts, wasps should lay all males in the small hosts and all females in the medium hosts. But if a host population contains 50% medium-size hosts and 50% large hosts, the wasps should lay all males in the medium hosts and all females in the large hosts. The medium-size hosts

receive either males or females depending on whether they occur with large or small hosts.

Materials and Methods

To test these predictions and assumptions I conducted experiments with laboratory populations of the host (*Callosobruchus chinensis*) and parasitoid (*Heterospilus prosopidis*) in 15 × 100 mm plastic petri dishes, each containing 20 g of Azuki beans (about 200 beans). All cultures were kept in environmental chambers at 30° C and 70% relative humidity. I exposed all or a portion of each dish's beans for 24 h to 30 to 35 host adults per 20 g of beans at intervals depending on the particular experiment. This provided one to two host eggs per bean. When host larvae reached the desired age I introduced 15 female and 10 male wasps per dish. After 24 h I removed the wasps and counted the sex ratios as the progeny emerged.

Superparasitism was kept to a minimum in these experiments. Densities were controlled at about 20 host eggs per female wasp, easily more than the wasps could exploit in 24 h (Kistler 1979). Within a dish all wasps had access to all hosts, and since *H. prosopidis* are effective at finding unused hosts (Utida 1957), attempts by different females to lay eggs on the same host were probably infrequent.

Results

To determine if *H. prosopidis* did in fact skew offspring sex ratios as a function of host size, I presented wasps with hosts aged 11, 13, 15, 17, and 19 days. In this experiment I kept host age classes separate so that no wasp encountered mixed age classes. The results are shown in Fig. 1. In four of the five host age classes the sex ratios were significantly different from one. The proportion of males was inversely related to host age, with the most dramatic decrease occurring over the 11 to 15 day host age interval.
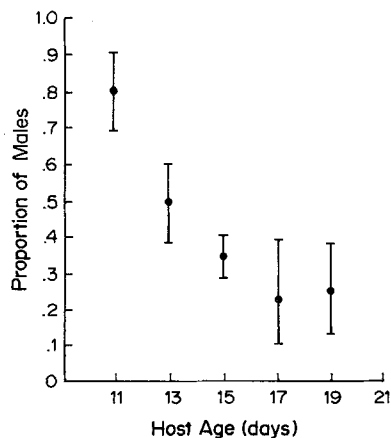

Fig. 1. Proportion of males emerging from various host ages. The *vertical bars* show the 95% confidence limits. (*Z* test) The pairs of host ages producing significantly different wasp sex ratios are: 11 and 13, 11 and 15, 11 and 17, 11 and 19, 13 and 17, 13 and 19 (two-tailed *Z* test)

Table 1. Combined mortality of wasps, host larvae, and host eggs. Mortality is measured as the percentage of host eggs that produce neither host nor parasitoid. The difference between the number of host eggs and the number of hosts emerging in the predator-free group equals the mortality of host eggs and larvae. In the three experimental groups the difference between the number of host eggs and the number of emerging individuals of both species equals the combined mortalities of host eggs, host larvae, and wasps

Host age	Number of host eggs	Number of hosts emerged	Number of wasps emerged	Mortality
11 day	785	383	162	30.6%
15 day	801	250	281	33.7%
19 day	872	546	122	23.4%
No predation	938	748	–	20.3%

These results imply either that females adjust the sex ratio of eggs, or that wasps lay mostly fertilized eggs in all host size classes, but female larvae suffer high mortality in smaller hosts so that mostly males emerge from these host sizes. To see if the latter was occurring, I exposed host populations to the wasps at 11, 15, or 19 days, and compared these with a parasitoid-free control. I counted the number of host eggs in each group and the numbers of hosts and parasitoids as they emerged. By comparing the combined mortality of wasps, hosts, and eggs in the three groups with parasitism to the host and egg mortality in the parasitoid-free group, the mortality of the wasps was estimated. The results are in Table 1. Mortality varied somewhat from group to group, probably due to differences in host density, but this variation was not consistent with the possibility that skewed sex ratios resulted from differential mortality between sexes. If that were the case, the 11-day host group would have had greater mortality than the 15-day host group. Since this did not occur, the sex ratio differences between host age classes must be due to differences in ratios of fertilized to unfertilized eggs laid by the wasps.

Based on the relative fitnesses of males and females in small and large hosts, Charnov's model predicts how wasp sex ratios in a particular host size should change as the host size distribution changes. To test these predictions, I presented wasps with small (11-day old) and large (15-day old) hosts in various proportions. The results are shown in Table 2. In this case the model predicts that as the proportion of 11-day hosts increased, the proportion of males in 11-day hosts should have remained at 1, and the proportion of males in the 15-day hosts should have increased from 0 to about 0.5. In the 11-day hosts the results were fairly close to the predictions, but not in the 15-day hosts. Instead of increasing from

Table 2. Proportion of male offspring when the parents are presented with various combinations of small (11-day old) and large (15-day old) hosts. Sample sizes are in parentheses

	11-day hosts	15-day hosts
90% 11 day: 10% 15 day	0.82 (226)	0.22 (23)
70% 11 day: 30% 15 day	0.91 (141)	0.43 (103)
50% 11 day: 50% 15 day	0.85 (20)	0.32 (224)
30% 11 day: 70% 15 day	1.0 (29)	0.35 (185)
10% 11 day: 90% 15 day	0.82 (33)	0.36 (232)

Table 3

A. Fitness of females raised on 11, 15, and 19-day old hosts

Host age	Mean number of progeny per female ± S.D. (n)
11 day	1.3 ± 2.9 (61)
15 day	27.4 ± 8.4 (46)
19 day	22.8 ± 6.0 (45)

B. Fitness of males raised on 11, 15, and 19-day old hosts

Host age	Mean number of females fertilized in 3 h ± S.D. (n)
11 day	2.8 ± 2.4 (38)
15 day	8.3 ± 2.4 (31)
19 day	7.5 ± 2.3 (35)

Table 4. Proportion of male offspring whose parents received small and medium size hosts or medium and large hosts

Host age	Males	Females	Proportion of males
Wasps received 11 and 15 or 15 and 19-day hosts			
11 days and	94	19	0.83
15 days	113	239	0.32
15 days and	121	245	0.33
19 days	22	66	0.25
Wasps received 11 and 13 or 13 and 15-day hosts			
11 days and	17	3	0.85
13 days	39	72	0.35
13 days and	40	82	0.33
15 days	7	10	0.41

0 to 0.5, the male proportions in the 15-day hosts remained constant at the same proportion that occurred when wasps are presented with only 15-day hosts. The variation that did occur was probably due to the small size of several samples. Thus the model's predictions were not fulfilled for this wasp species.

To learn why the wasps did not conform to the model, I reexamined the model's two assumptions to see if they hold true for this species. The first assumption is that wasp fitness increases with host size and that the effect is more pronounced in females than in males. To test this assumption, I compared the fitness increases for males and females from small to large hosts. I raised female wasps on hosts aged 11, 15, and 19 days and then individually isolated them for their entire adult lives with one male and a superabundant host supply. The number of progeny for each individual was then a measure of lifetime fecundity. I raised male wasps on hosts aged 11, 15, and 19 days, and then presented each with 15 virgin females for three hours. These females were then individually isolated with a host supply and allowed to lay eggs so that the number of females with female offspring was an indication of the number of fertilizations achieved by each male. The results are shown in Table 3. Female fitness increased 21-fold from the

11 to the 15 day old hosts, while male fitness increased only 3-fold.

This estimate of male fitness as it stands is inadequate in an important way. While the estimate of female fitness measures lifetime fecundity, male fecundity is measured only over a three hour period, and the effects of longevity on male fitness are not assessed. Longevity is an important component of male fitness here since wasps from 11-day old (small) hosts lived about two days and wasps from 15-day old (large) hosts lived a week or at most ten days. If I take a 5-fold increase as an estimate of the change in male longevity from small to large hosts, then the increase in male fitness from 11 to 15 day old hosts becomes 15-fold. This is still less than the 21-fold increase in female fitness. It is over this same host age interval that the sex ratios were reversed (see Fig. 1). Thus the first assumption of the model seems valid for this wasp species, at least as far as this test can discern.

The second assumption is that female wasps estimate host size distributions and adjust offspring sex ratios to maximize their fitness. To test this assumption I presented one group of wasps with hosts aged 11 and 15 days in equal numbers and a second group with hosts aged 15 and 19 days. (I also repeated this experiment using 11, 13, and 15 day old hosts.) If the wasps were able to make the assumed sex ratio adjustment, then would expect predominately female offspring in the 15 day old hosts of group 1, but mostly males in the 15 day hosts of group 2. Table 4 shows that this prediction was not fulfilled. Instead the wasp sex ratios in the two groups of 15 day old hosts were virtually identical, and also very close to the sex ratio when the wasps received 15 day old hosts alone in the first experiment. Thus the second assumption of the model does not hold for *H. prosopidis*. These wasps do not adjust offspring sex ratios to the host size distribution they encounter, but instead

they lay the same ratio of fertilized to unfertilized eggs in a particular size host regardless of what other host sizes are present.

Discussion

One might argue that the wasps were unable to assess the numbers of large and small hosts and therefore were unable to adjust offspring sex ratios to host distributions because they were exploiting an unnatural host species, though they may still have these abilities in a natural situation. This seems unlikely, however, since the wasps did distinguish large from small hosts. It is more probable that *H. prosopidis* females simply do not make this sex ratio adjustment. But it still possible that selection has adjusted their sex ratios to host size distributions. Instead of being adjusted to the host size distributions encountered by individual females, the sex ratio may be attuned to the average host size distribution encountered over many generations. This sort of multiple generation process I shall refer to as a long term sex ratio adjustment, while the process assumed by the model will be called a short term adjustment.

While *H. prosopidis* did not make the short term adjustment, other wasp species do have this ability (Chewyreuv 1913; van den Assem 1971). This leads to the question of why some species have evolved this ability and others have not. One possible answer is that the necessary genes or gene complexes may not have occurred in some species. Pleiotropism involving adverse effects on other traits may limit a species potential for evolving this ability.

Another explanation concerns the host size distributions normally encountered in nature and the equilibrium sex ratio for a population of haplodiploid parasitoids. Even though males possess only half the chromosomes of females, the expected sex ratio in a solitary outbred species is 1 (Hartl and Brown 1970; Charnov 1979). When the sex ratio departs from 1, individuals over-producing offspring of the minority sex gain an advantage since their offspring will make a disproportionately large genetic contribution to future generations (Fisher 1930). In an outbreeding wasp species that does not make the short term adjustment the population sex ratio within a generation is dictated by the available host sizes. When the distribution of host sizes varies little from generation to generation of the wasps, their sex ratio should adjust in the long term manner to that distribution and not vary greatly from 1. In this situation there would be no significant advantage in making the short term sex ratio adjustment.

Suppose, however, that the host size distributions encountered by successive wasp generations varied a great deal so that sometimes small hosts predominated while at other times there were mostly large hosts. If a species capable only of long term adjustments were faced with this situation, its population sex ratio would vary along with the host size distribution; some generations would consist primarily of males, others largely of females. Now suppose that a few individuals acquired the ability to estimate host size distributions and lay more of the minority sex than the host sizes dictated. These individuals would have increased fitness over the rest of the population since their offspring would, on the average, make a larger genetic contribution than offspring of parents without this ability. Gene combinations imparting this ability should therefore increase in relative frequency. Thus a parasitoid species that encounters host size distributions changing little over time should make only the long term multiple generation adjustments in its sex ratio strategy, but species that are faced with wide variation in host size distributions should have the ability to make short term single generation adjustments. Whether this is actually so can be determined from studies of the variation in host size distributions found in nature combined with experiments like the host size switching experiment (Table 4) described here.

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