

Oviposition strategy of the parasitic wasp *Dinarmus basalis* (Hymenoptera, Pteromalidae)

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Summary

Host type choice and sex allocation were examined using the solitary parasitic wasp *Dinarmus basalis* (Pteromalidae, Hymenoptera) parasitizing larvae or pupae of the bean weevil *Callosobruchus chinensis* (Bruchidae, Coleoptera) within azuki beans (*Vigna angularis*). The wasps were offered two types of host; one was hard for the mother to lay eggs in, but was more beneficial for the offspring; the other was easy for the mother to lay eggs in, but was less beneficial for the offspring. The two types of host were one large host (17-day old host) in one bean and 6 small hosts (12-, or 13-day old hosts) in one bean. The same number of each host was presented at the same time to female wasps. The wasps accepted more 17-day old hosts than 12-day old hosts, and more 13-day old hosts than 17-day old hosts in each pair-wise choice experiment. The proportions of accepted host types were different from the proportions predicted by optimization models of random prey encounter with known or unknown prey densities. The wasps showed partial preference of host types. Incomplete information about prey densities, and about the costs and benefits of the two types of host may have generated the partial preference. Two predictions of host size-models, that (1) there should be a negative relationship between host size and offspring sex ratio (proportion of male offsprings), and (2) the sex ratio in each size host changes with the relative frequency of each size host utilized, were qualitatively supported.

Keywords: solitary wasp; host choice; sex allocation

Introduction

Host choice models (which correspond to a diet choice model, e.g. Emlen, 1973; Charnov, 1976; Shoener, 1971; Stephens and Krebs, 1986) present an explanation of offspring allocation among different host types. Host-size models (e.g. Charnov, 1979) present an explanation of offspring sex allocation in different size hosts under a given frequency of host size distribution utilized by the female in parasitic wasps.

Suppose there are some types of host that differ in quality: to accept or reject a host is an important decision problem for female wasps. If a female wasp can find enough high quality hosts relative to the number of eggs which can be laid, then she might reject any inferior hosts that she encounters. On the other hand, if she does not encounter enough high quality hosts on which to lay her eggs, the host utilization pattern depends on two factors: knowledge of prey densities, and the fecundity of the female wasp. Under the assumption of incomplete information of prey densities, optimality theory predicts the on-off rule (a host should either always be accepted or always rejected upon encounter (see Stephens and Krebs, 1986)). This means that if the wasp accepts both superior and inferior hosts when there are a limited number of superior hosts, it may utilize both superior and inferior hosts in proportion to encounter rates. Furthermore, if the wasp has complete information of prey densities but a limited fecundity, she

should lay as many eggs as possible on superior hosts. Discrimination among different quality hosts and accurate information on the availability of each host type are important for optimal behaviour in a given environment.

Many parasitoid wasps are known to adjust sex allocation of offspring under various environmental conditions (van den Assem, 1971; van den Assem *et al.*, 1984; Charnov *et al.*, 1981; Jones, 1982; Waage and Lane, 1984; Waage and Ming, 1984; King, 1987, 1988, 1989, 1990, 1991; Werren and Simbolotti, 1989). The fitness of an ovipositing female is influenced by the allocation of male and female eggs on different size hosts under a given level of local mate competition (Charnov, 1979; Charnov *et al.*, 1981; Werren, 1984; Werren and Simbolotti, 1989). This idea has been referred to as host-size models for solitary parasitoid wasps (Charnov, 1979; Charnov *et al.*, 1981). Host-size models qualitatively predict that for solitary species of parasitoids, mothers should oviposit a greater proportion of the sex that has larger relative fitness in a given host size (Charnov, 1979, 1982; Charnov *et al.*, 1981). For most species of parasitoid wasps examined, there is a negative relationship between proportion of sons and host size (e.g., Charnov *et al.*, 1981; King, 1988, 1990; Fujii and Kihn Mar Wai, 1990). These results suggest that developing on a larger host confers more to the ultimate reproductive success of females than to that of males. Charnov *et al.* (1981) further predicted that the allocation of sex to a particular host-size class depends on the relative frequency with which other host sizes are utilized.

This paper addresses two separate questions by using a solitary parasitoid wasp (*Dinarmus basalis*) parasitizing weevil larvae and pupae within beans: (1) whether the wasp manipulates egg allocation between two alternative choices of host types according to the prediction of the theory of optimal host choice (Stephens and Krebs, 1986), and (2) whether the wasp's manipulation of offspring sex ratio in response to host size and its utilization of the alternative size class of host is consistent with the host-size model (Charnov, 1981).

Materials and methods

The experiments were conducted in a growth cabinet controlled at 30 °C, 70% RH and 24 L. *Dinarmus basalis* (Pteromalidae, Hymenoptera) is an ectoparasite on larvae and pupae of several species of grain and bean weevil. This work used the azuki bean weevil *Callosobruchus chinensis* (Bruchidae, Coleoptera) infesting azuki beans (*Vigna angularis*) as the host species in these experiments.

The parasitic wasps tend to avoid superparasitism (Khim Mar Wai and Fujii, 1990). However, if superparasitism occurs, the wasps exhibit contest type competition in the developing stage and, therefore, only one adult wasp emerges from one host (Khim Mar Wai and Fujii, 1990). The ages of hosts used in the experiments were 12-, 13-, and 17-day old since oviposition by mother weevils. Twelve-, and 13-day old hosts are larvae, and 17-day old hosts are pupae. Host weight correlates with the age among the three age classes.

To set host choice situations with the same host species, high aggregations were created for smaller hosts (12-, or 13-day old hosts) and sparse aggregations for large hosts (17-day old hosts) in one bean (details given before). When there are many hosts in a bean, the bean is moistened and the texture of the bean is softer because of the respiration of the hosts. The aggregated hosts are superior in terms of oviposition, but inferior for offspring oviposited. On the other hand, the sparse hosts are inferior in terms of oviposition, but superior in terms of resources for offspring oviposited. Therefore, the small size of the young age hosts may be compensated by the host aggregation making them more available to the mother wasps.

We can easily calibrate the number of host in one bean. An arbitrary number of inseminated

adult female bean weevils was placed in a Petri dish with azuki beans. After about 0.5–1 h the weevils were removed from the dish. Some eggs were laid on the surface of the beans. The average number of eggs on the surface of one bean can be controlled roughly by the density of introduced adult female bean weevils. The dish was kept until the larvae of the weevils hatched from the eggs after three days in the growth cabinet. Hatched eggs are easily identified from unhatched eggs by their color. Beans were selected that had the fixed numbers of hatched eggs. After hatching, the mortality rates of larvae and pupae of the weevil are extremely low under the conditions of the experiment (unpublished data).

No-choice experiments

Three main no-choice experiments and one supplementary experiment were performed. Five azuki beans, each infested by 6 bean weevils (12- or 13-day old hosts), and 25 uninfested beans were placed in one compartment of a four-compartment Petri dish (Falcon No. 1009, 100 mm diameter, 15 mm depth). The experiments are denoted as NoCh12 and NoCh13, respectively. In the third experiment, thirty azuki beans, each infested by one weevil (17-day old host), were placed in one compartment of a four-compartment Petri dish (NoCh17). In the supplementary experiment thirty azuki beans, each infested by six 17-day old hosts were placed in one compartment of a four-compartment Petri dish (NoCh17H).

Newly emerged inseminated female parasitoids from a stock culture were separately introduced into a dish. The female seeks hosts that are located inside the beans from the surface of the beans, and oviposits eggs on the hosts. The female was transferred into each dish with hosts and kept for 24 h; every 24 h, the female wasp was transferred into a new system. The transfer was continued until the female wasp died. Each experiment was replicated six times.

Choice experiments

Two choice experiments were performed. In the first experiment, five beans, each infested by six 12-day old hosts and 25 uninfested beans were placed in one compartment of a dish. Thirty beans infested by one 17-day old host were placed in the opposite corner of the dish (Ch12/17). In the second experiment, five beans infested by six 13-day old hosts each and 25 uninfested beans were placed in one compartment of a dish. Thirty beans infested by one 17-day old host were placed in the opposite corner of the dish (Ch13/17). Solitary inseminated females were introduced into each dish as described above (see No-choice experiments).

Results

Host quality

Host weights among the three host age classes were compared, and 50 individual hosts of each age were weighed using hosts that were at the same densities in the beans as the hosts in the main experiments. Mean host weights were different among age classes ($F_{2,147} = 272.305$, $p < 0.0001$) (Table 1). Fisher's *post hoc* LSD test showed that all the pairs of age classes are

Table 1. Mean weight of host in each age class.

Age class	Weight (mg)	SD	<i>n</i>
12	1.607	0.775	50
13	5.151	1.434	50
17	6.823	1.119	50

Table 2. Mean number of offspring emerging from the hosts in first 6 days in each experiment.

Experiment	Number of offspring	SD	<i>n</i>
NoCh12	59.50	8.983	6
NoCh13	65.50	10.521	6
NoCh17	79.00	2.280	6
NoCh17H	89.33	9.459	6
Ch12/17	85.00	16.248	6
Ch13/17	83.00	12.296	6

significantly different from each other at the type I error level of 0.0001. These results show that host age is a good predictor of host weight.

Numbers of offspring

The numbers of offspring from the hosts that were parasitized by the mother in her first 6 days in each experiment are shown in Table 2. The wasps take about 5–10 min to lay eggs after finding a host from the surface of the infested bean (personal observation). When hosts are abundant, the female wasp can lay all the eggs she accommodates. On 17-day old hosts, there is very little mortality during the larval and pupal stage of the developing wasp (unpublished data); thus, in NoCh17H, we can assume that the number of offspring emerged approximately equals the number of eggs oviposited. It was assumed that, in NoCh17H, there were abundant hosts and, therefore, this estimates the maximal production of eggs.

The mean number of offspring among all experiments was compared using a one way ANOVA. The means among experiments were statistically different ($F_{5,30} = 7.131, p < 0.0002$). *A priori*, it is hypothesized that the female wasp cannot realize the maximal production of eggs in NoCh12, NoCh13, and NoCh17, but can realize the maximal production of eggs in NoCh17H, Ch12/17, and Ch13/17. Therefore, two planned comparisons were conducted: (NoCh12, NoCh13, NoCh17) versus (NoCh17H, Ch12/17, Ch13/17); and (NoCh17H) versus (Ch12/17, Ch13/17). In the former analysis, there was a significant difference between the two groups ($F_{1,30} = 24.337, p < 0.0001$). In the latter analysis, there was no significant difference between the two groups ($F_{1,30} = 0.973, p < 0.3317$). Although the data set did not satisfy homoscedasticity, the *p* value of the former analysis was extremely small: therefore, it was concluded that female wasps did not realize maximal production of eggs in NoCh12, NoCh13 and NoCh17; but realized maximal production of eggs in Ch12/17 and Ch13/17.

Allocation of offspring between two host types

In the choice experiments, the variance in the proportion of offspring that emerged from 17-day old hosts was compared between the two choice experiments (Ch12/17 and Ch13/17). The proportions were normalized by a square root arcsine transformation. The variances did not differ ($F_{2,5} = 3.2, p < 0.25$). The variances of the proportion of larger hosts utilized among replicates in each choice experiment were not different.

According to the classical theory of optimal diet choice (Shoener, 1971; Stephens and Krebs, 1986), if the superior hosts (evaluated by the expected fitness per unit time invested) are in limited supply, the wasps should lay eggs on both types (superior and inferior types) of host. Otherwise, if the higher rank hosts are abundant, the wasps should utilize these only. In the former case (no choice situation), it can be predicted that offspring emerging from each type of

host in the choice experiments are proportional to the offspring numbers in no-choice experiments. Following the null prediction (no choice), the offspring allocations in choice experiments are equal to the ratios of the number of emerging offsprings in no-choice experiments (NoCh12/NoCh17 and NoCh13/NoCh17).

To compare the null mean ratio and the observed mean ratio of offspring emerging from smaller hosts to those from larger hosts in the choice experiments, null ratio data sets were generated by computing all possible combinations of the six values of offspring numbers emerging from small hosts (NoCh12 or NoCh13) and the six values of offspring numbers emerging from large hosts (NoCh17). The null data sets are Null12/17 and Null13/17, each of which consists of 36 ratios. The mean ratios of Null12/17 to those of Ch12/17, and the mean ratios of Null13/17 to those of Ch13/17 were compared using the Mann Whitney U test. There were significant differences between Null12/17 and Ch12/17 ($z = -2.59$, $p < 0.0096$), and Null 13/17 and Ch13/17 ($z = -2.804$, $p < 0.005$).

When x and y are independent random variables, the mean of the ratio x to y (x/y) is

$$E \left[\frac{x}{y} \right] \approx \frac{\mu_x}{\mu_y} + \frac{\mu_x \sigma_y^2}{\mu_y^3} \quad (1)$$

(Welsh *et al.*, 1988). Based on the no-choice experiments, the null expectation of the ratio of offspring emerging from 12-day old hosts to those from 17-day hosts (12-day old hosts/17-day old hosts) is 0.749. Similarly, the estimated expected null ratio of offspring emerging from 13-day old hosts to those from 17-day old hosts (13-day old hosts/17-day old hosts) is 0.8056. The ratio in each choice experiment is 0.400 in Ch12/17 and 2.822 in Ch13/17. This leads to the conclusion that the female wasps lay relatively more eggs in larger hosts (17-day hosts) in Ch12/17, and in smaller hosts (13-day hosts) in Ch13/17 than predicted by null models.

Sex allocation in each host type

An analysis was made of whether the wasps changed their sex allocation in each host type according to the presence of the other type of host, and the proportion of male offspring produced in each age host class was compared between choice and no-choice experiments.

There was a significant difference in sex ratio between the 12-day old hosts of NoCh12 and Ch12/17, and between the 17-day old hosts of NoCh17 and Ch13/17 (see Table 3). In 12-day old hosts the proportion of males is larger in the choice experiment (Ch12/17) than in the no-choice experiment (NoCh12) (Fig. 1). In 17-day old hosts the proportion of males is lower in the choice experiment (Ch13/17) than in the no-choice experiment (NoCh17) (Fig. 1).

The weights of adult wasps emerging from each age host

The mean weights of adults of each sex which emerged from each host were compared. For each

Table 3. F and P values in a comparison of sex ratios. Square root arcsine transformation of sex ratios was conducted to generate normality of the data distributions.

Source	$F_{1,10}$	p
12-day host (NoCh12 vs Ch12/17)	6.610	0.0278
13-day host (NoCh13 vs Ch13/17)	0.037	0.8518*
17-day host (NoCh17 vs Ch12/17)	3.595	0.0872*
17-day host (NoCh12 vs Ch13/17)	7.721	0.0195

*Not significant

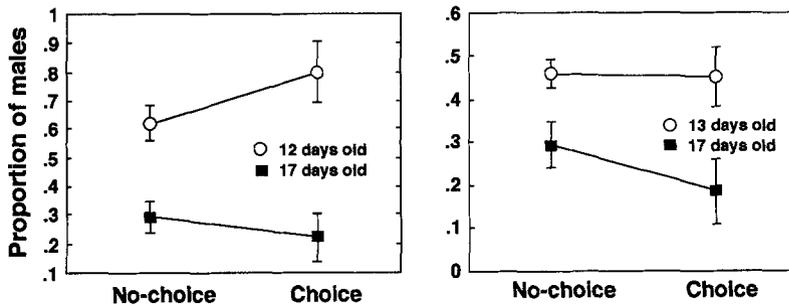


Figure 1. Sex ratios of offspring in each host type in each experiment. Vertical bars show the standard deviations.

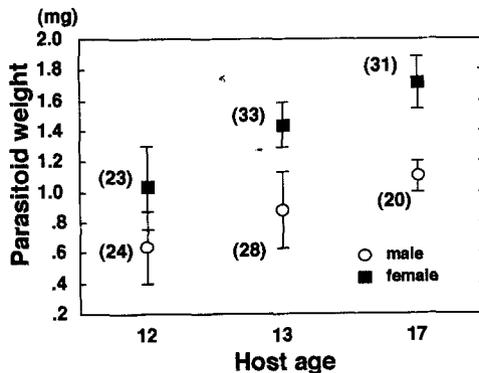


Figure 2. Mean weights of males and females emerged from three age classes of host. Vertical bars show the standard deviations. The numbers of samples are in parentheses.

sex, there are statistically significant differences among the means of weights ($F_{2,84} = 80.481$, $p < 0.0001$ for females; $F_{2,69} = 26.00$, $p < 0.0001$ for males). Fisher's *post hoc* LSD tests showed that all the pairs in each sex are significantly different from each other (at the level 0.0001, respectively). Average weight increased with host age and hence host weight in both sexes (Fig. 2).

Discussion

The wasps in NoCh12, NoCh13, and NoCh17 laid fewer eggs than those in NoCh17H. In the unconstrained condition (NoCh17H), the wasps laid about 15 eggs per day. Since the number and density of each host type in the choice experiments were the same as those in the no-choice main experiments (NoCh12, NoCh13, and NoCh17), the wasps in the choice experiments could not lay all their eggs in one type of host. From the view point of optimality theory, if the female wasp knows the abundance of the two types of host in the Petri-dish, she should lay as many eggs as possible on the higher ranking hosts. On the other hand, if the female does not know the abundance of the both types of host in the Petri-dish, she should lay her eggs in proportion to the encounter rate of the two types of hosts. The wasps did not show the patterns of the above possibilities, but exhibited partial preference of host type in choice experiments. This suggests

that the wasps have incomplete knowledge of the prey densities and the number of eggs to be laid.

Both the relative host size and the aggregation pattern of the hosts determined the relative value of the host types. The wasps may choose hosts by the cost of oviposition on the host and the benefit given that an egg is oviposited on the host. Neither the actual cost of oviposition nor benefit for offspring on each host type was quantified. The evidence that the fitness of the emerging offspring changes with host age is indirect. The mean sizes (weights) of adult males and females emerging from each age host differed (Fig. 2). If fitness of offspring is related to body weight in both males and females, the fitness of the mother wasp will increase as host age increases. However, the time and energy costs of oviposition may distort the choice preference. In the choice experiments, the wasps chose more 17-day old hosts than 12-day old hosts in Ch12/17, and more 13-day old hosts than 17-day old hosts in Ch13/17. This may be a consequence of the choice between the cost of ovipositing eggs and the benefit through individual offspring from each age class of host. Based on the pairwise choice experiments, the hosts may be ranked from 13-, to 17-, to 12-day old host, in descending order.

The primary sex allocation on each host age cannot be evaluated by this study. Larval mortality may or may not differ between males and females on the younger age class of the host (Fujii and Khin Mar Wai, 1990; Moriguchi, 1992). But here the focus was not on the primary sex ratio on each host age but on the change in sex ratio with the proportion of hosts utilized in two age classes in each choice experiment. Host size models suggest that the sex ratio in each host size depends on the relative frequency of each size of host utilized by the wasp (Charnov, 1979; Charnov *et al.*, 1981). The host size model (Charnov, 1979, p. 477) predicts the following: when small hosts or large hosts are presented separately to wasps, the equilibrium sex ratios are the same. When one host type is rare in a situation where two types of host are utilized, the sex ratio on the common host type is still around the equilibrium, but the sex ratio on the rare host type becomes zero (on large hosts, i.e., all are females) or unity (on small hosts, i.e., all are male). The results here correspond partly to the prediction of Charnov's host size model (1979). In Ch12/17, the wasps utilized many 17-day old hosts and few 12-day old hosts, and the sex ratio on 17-day old hosts did not differ from the sex ratio in the no-choice experiment (NoCh17), but the sex ratio on 12-day old hosts increased compared with the sex ratio in the no-choice experiment (NoCh12). In Ch13/17, the wasps utilized many 13-day hosts and few 17-day hosts, and the sex ratio on 13-day old hosts did not differ from the sex ratio in the no-choice experiment (NoCh13), but the sex ratio on 17-day old hosts decreased relative to the sex ratio in the no-choice experiment (NoCh17). The sex ratio changed on the less utilized hosts. Similar sex ratio shifts were also observed by Charnov *et al.* (1981) and King (1990).

In the experiments described here, female parasitoids encountered two decision steps: which type of host to utilize and what sex ratio of eggs to lay. In conclusion: (1) the wasps ranked host types; (2) the wasps exhibited partial preference; (3) the offspring sex ratio was adjusted on rare host types in the choice situation. To understand the pattern of host choice and sex allocation of the parasitic wasps more thoroughly, we have to know oviposition cost and progeny fitness, given the oviposition. Oviposition cost may depend not only on the energy and time cost of ovipositing eggs but also the state of the wasps (e.g., remaining eggs and energy or life span). To measure the progeny fitness is another difficulty. Fitness changes related to body size within gender may or may not be different between the two sexes (Charnov *et al.*, 1981; Heinz, 1991; Hurlbutt, 1987; King, 1988). Furthermore, the fitness of male and female individuals depends on the frequency of the two sexes (Werren and Simbolotti, 1989). A model which incorporates these factors (size- and frequency-dependent progeny fitness, and state of mother) is needed to understand the pattern of the host choice and sex allocation strategy of parasitic wasps.

Acknowledgments

I gratefully acknowledge the useful comments of Bethia King and Derek A. Roff. I also thank Koichi Fujii for his encouragement and support. This work was supported by the Tsasakawa Scientific Research Grant from the Japan Science Society and University of Tsukuba Project Research Funds.

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