Host selection by virgin and inseminated females of the parasitic wasp, *Dinarmus basalis* (Pteromalidae, Hymenoptera)

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Summary

1. Fitness is related to reproduction and survival. There apparently exists a negative correlation between the numbers of male and female offspring. There also exists a trade-off between survival and reproduction. This paper investigates optimal decisions with the reproduction and survival trade-off in host selection by wasps.

2. Whereas inseminated female wasps could manipulate the sex of their offspring, virgin females produced only male offspring. I surveyed behavioural differences and the consequences of oviposition by inseminated and virgin females of a solitary parasitic wasp in host choice situations.

3. Two host types were available at the same time to both inseminated and virgin female wasps: one (a 17-day-old host in one bean) presenting difficulties for the laying of eggs, but more benefits for the offspring and the other (five 12- or 13-day-old hosts in one bean) easier for the female wasp for laying of eggs but less beneficial for the offspring.

4. Inseminated female wasps chose more 17-day-old hosts than 12-day-old hosts, but more 13-day-old hosts than 17-day-old hosts in each pair-wise choice. Virgin females chose the smaller hosts in both situations.

5. Virgin females, having greater longevity than inseminated females, laid larger numbers of eggs than the inseminated females during their lifetime by adopting an energy-saving host choice that had little effect on male offspring fitness.

Key-words: Host choice, insemination status, solitary parasitic wasp

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Introduction

Parasitic wasps are useful for investigating decision-making in reproductive behaviour. During oviposition, the female wasp must make a series of well-defined decisions that have major consequences for her lifetime fitness. Females that are mated can manipulate the sex of the offspring; unfertilized eggs develop into males and fertilized eggs into females. The decisions determining sex allocation by mated female wasps in certain environmental conditions have been well studied, providing fruitful insights into fitness maximization behaviour in the evolutionary process (Hamilton 1967; Charnov 1979; reviewed in King 1987, 1993). Modification of several aspects of the decision processes, such as clutch size and host acceptance, determine the direction of sex manipulation (Charnov 1979; Suzuki & Iwasa 1980; Werren 1980, 1984; King 1988; Werren & Simbolotti 1989; Nishimura 1993).

Since it has been assumed that the ovipositing female wasps have already been inseminated (reviewed in Godfray & Hardy 1993), the oviposition strategies and sex allocation offspring of mated females have been of primary concern (Hamilton 1967; Werren 1980, 1983; Charnov *et al.* 1981; King 1989). On the other hand, in evolutionary scenarios, it is unclear whether or not virgin females should lay eggs or postpone reproduction until after mating (Godfray & Grafen 1987; Godfray 1988, 1990; Antolin 1989; Godfray & Hardy 1993). Because the offspring from a virgin female comprise only males, decision to oviposit eggs as a virgin depends on various external and internal factors (Godfray & Hardy 1993).

Given that virgin female wasps oviposit eggs, there are still interesting biological problems. Life-history parameters, such as fecundity, longevity and reproductive behaviour of virgin females can be compared with those of mated females (e.g. Antolin 1989; Donaldson & Walter 1991). Because a virgin female is constrained in its behavioural decisions regarding the sex allocation of offspring, other

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decision processes might be affected. Consequently, the life-history parameters may change from those of inseminated females.

A virgin female wasp may not remain so indefinitely. In this paper, however, only the oviposition strategy of constrained (virgin) females is considered; subsequent copulation strategies of females are not considered.

Selection may favour female wasps that lay their eggs on hosts providing high survival and reproductive success of the offspring, irrespective of insemination status. They may also be selected for longevity, thus laying many eggs over the greater time available. However, a trade-off exists between reproduction and survival (Goodman 1979, 1982; Bell & Koufopanou 1986; van Noordwijk & de Jong 1986; Roff 1992; Stearns 1992; Perrin, Sibly & Nichols 1993).

The host choice pattern in ovipositing parasitic wasps should involve two factors: a within-generation factor and a between-generations factor. The ovipositing female will try to lay as many eggs as possible, at the same time minimizing energy used for oviposition. On the other hand, concern for the offspring may also occur, since host condition (size and quality) affects the fitness (growth and survival) of offspring. The balance between these factors may differ between constrained and unconstrained ovipositing wasps.

In this paper, the manner in which insemination status affects host choice behaviour and life-history parameters is examined, using a solitary parasitic wasp (*Dinarmus basalis*, Pteromalidae, Hymenoptera) parasitizing Azuki Bean Weevil (*Callosobruchus chinensis*, Buruchidae, Coleoptera) larvae and pupae within Azuki Beans (*Vigna angularis*). The wasp is a synovigenic species (producing eggs throughout its life), ectoparasitic on larvae and pupae of several species of grain and bean weevils.

Host condition affects the fitness of both male and female offspring (i.e. host mass correlates with the size of emerging offspring wasps in both sexes) (Nishimura 1993). The relative change in fitness with change in host size is greater for females than for males (Nishimura 1993). In *D. basalis*, unconstrained (inseminated) females lay more fertilized eggs (female) on larger hosts and more unfertilized eggs (male) on smaller hosts, if both host types are available (Nishimura 1993). This shows the optimal (most efficient) sex allocation within each host type given that a certain number of both are utilized.

When many hosts occur in a bean, the bean is moistened and the texture softened owing to the respiration of the hosts inside the bean, even if the hosts are small (Nishimura 1993). Small aggregated hosts are advantageous for oviposition, but disadvantageous for the subsequent offspring. On the other hand, large sparsely distributed hosts are disadvantageous for oviposition, but advantageous (owing to the greater overall) for the offspring. Therefore, the size of smaller hosts may be compensated by their host aggregation, making them more easily available to ovipositing females.

In this study, ovipositing female wasps were provided with the choice of saving energy by laying eggs on small hosts, or spending greater energy by laying eggs on large hosts. If the females laid eggs according to their virgin or inseminated condition, a difference in host choice pattern so as to maximize fitness should be evident. The study focused on whether or not choice behaviour differed between virgin and inseminated females, and how such differences might affect longevity and fecundity of the wasps. It was predicted that virgin females would utilize small aggregated hosts, the consequent saving of energy leading to increased longevity and greater opportunities for egg laying.

Materials and methods

The experiments were conducted in a growth cabinet maintained at 30 °C, 70% RH and 24 l, using the Azuki Bean Weevil infesting Azuki Beans as the host species. Female *D. basalis* insert the ovipositor through the bean surface, laying an egg on a host larva or pupa, if located. Before depositing the egg, the wasp anaesthetizes the host and feeds by partially sucking out body fluid from the host. The anaesthetized host subsequently stops growing. The wasps tended to avoid superparasitism in high densities.

The ages of hosts used in the experiments were measured as 12, 13 and 17 days after oviposition by mother weevils. Twelve- and 13-day-old hosts are larvae, and 17-day-old hosts are pupae. Host mass positively correlates with age in the three age classes. The mean host mass is 1·607 mg (12-days old), 5·151 mg (13-days old) and 6·823 mg (17-days old), respectively (Nishimura 1993).

LONGEVITY OF INSEMINATED AND VIRGIN FEMALES

Inseminated and virgin female wasps (six individuals of each) were placed in empty Petri dishes individually and maintained in the growth cabinet until the wasps died. The longevity of each wasp was recorded.

SURVIVAL RATES OF WASP OFFSPRING ON DIFFERENT HOSTS

Because it was possible that survival rates differed between the host types on which eggs were laid, in addition to any differential mortality between male and female offspring possibly influencing the survival rates, the offspring from inseminated and virgin females laid on each host type were surveyed. One inseminated or virgin female wasp was placed in a dish with 12-, 13- or 17-day-old hosts for 24 h. The 12- and 13-day-old host dishes contained five beans with six hosts/bean and 25 host-free beans. The 17-day-old host dish contained 30 beans, each with a single host.

After 24 h, the female wasps were removed and the dishes kept for a further 2 weeks in the growth cabinet. Thereafter, the numbers of emerging wasp offspring and weevils were counted. Ten replicates were conducted for each insemination status of the parent wasps and for each host age class parasitized.

There was extremely low mortality of weevils (in high- and low-density conditions) not parasitized by wasps. Superparasitism was also found to be extremely rare. Since the parasitized host failed to develop further, the total number of hosts existing in a dish (30 individuals) minus the number of weevils emerging from the beans could be considered as the number of hosts parasitized (equals the number of oviposited eggs).

The survival rate (R) of wasp offspring was estimated in each replicate as:

$$R = N_{\text{wasps emerged}}/(30 - N_{\text{hosts emerged}}) \qquad \text{eqn 1}$$

where $N_{\text{wasps emerged}}$ is the number of wasp offspring emerging per dish, 30 is the total number of hosts offered, and N_{hosts} emerged is the number of hosts that have escaped parasitization.

CHOICE EXPERIMENTS

Five beans, each infested by 6-, 12- or 13-day-old hosts and 25 uninfested beans were placed in one compartment of a four-compartment Petri dish (100-mm diameter, 15-mm depth). Thirty beans, each infested by one 17-day-old host, were placed in the opposite compartment. Two choice experiments were prepared (12- *vs* 17-day-old hosts and 13- *vs* 17-day-old hosts). The choice types were encoded as Ch12/17 and Ch13/17.

The size of 12-day-old hosts might be compensated by their aggregation, thus equalizing sparsely distributed 17-day-old hosts in value for the ovipositing wasps. Since the size of 13-day-old hosts was close to that of 17-day-old hosts, the former were considered to be preferable. On that basis, it might be expected that wasps would choose 13-day-old hosts in Ch13/17, irrespective of insemination status. Thus, Ch13/17 was considered as a control experiment for Ch12/17. The two-way experimental design involved two factors: female status (inseminated or virgin) and choice condition (Ch12/17 and Ch13/17).

Newly emerged inseminated or virgin female wasps were introduced separately into a dish followed by 24-h isolation. Each female was then transferred to a dish with hosts and kept for 24 h. Every 24 h, thereafter, until death, the female wasp was transferred to a new dish. The wasp performed antenna search behaviour on the surface of the beans soon after placement in each dish. Upon finding a host below the bean surface, the wasp inserted the ovipositor into the bean and laid an egg on the host.

Beans with small and large hosts were placed into separate Petri dishes after 24-h parasitization and kept

for 2 weeks. The number of wasp offspring emerging subsequently from each host type was counted.

Choice type Ch12/17 was replicated five times with virgin females. Ch12/17 and Ch13/17 with inseminated females, and Ch13/17 with virgin females were each replicated six times.

Results

LONGEVITY OF INSEMINATED AND VIRGIN FEMALES

The longevities of inseminated and virgin females were compared. There was no significant difference between the two categories (inseminated: $8 \cdot 167 \pm 0 \cdot 401$ (SE) days; virgin: $8 \cdot 333 \pm 0 \cdot 760$ (SE) days; $U_{6,6} = 14 \cdot 5$, $P < 0 \cdot 575$). Thus, there was no apparent cost associated merely with insemination and sperm storage activity.

SURVIVAL RATE OF WASP OFFSPRING ON THE DIFFERENT HOSTS

A two-way ANOVA with factors, host type (age) and female wasp status (inseminated or virgin), was conducted to compare survival rates. Survival rates were transformed with an arcsine square root transformation.

There was a significant effect of host age on survival rate, but no significant differences between virgin and inseminated wasps (see Table 1). Data from the two wasp groups were pooled and Fisher's *post hoc* LSD test was conducted on host age to survey differences in survival rates among age classes. This indicated that the survival rates could be grouped (as 12- and 13-day-old hosts, and 17-day-old hosts) at the $0 \cdot 0001$ type I error level. Unbiased estimation of the survival rates of 12- and 13-day-old hosts: were $0 \cdot 491 \pm 0 \cdot 171$ SD ($n = 40$), and of 17-day-old hosts, $0 \cdot 851 \pm 0 \cdot 173$ SD ($n = 20$).

CHOICE EXPERIMENT

Two-way ANOVAs were conducted to survey differences in host utilization patterns. The factors are choice types (Ch12/17 and Ch13/17) and female wasp status (virgin or inseminated). In the analysis the effects of interest were the interaction (choice type × wasp status) and wasp status. When the interaction was significant, it alone was evaluated (i.e. the main effect (mate status) being ignored) (see Sokal & Rohlf 1981).

Table 1. ANOVA table of the effects of mating status and host age on the survival rate of *D. basalis* offspring

	df	MS	F	P
Host age	2	$0 \cdot 867$	$28 \cdot 152$	$0 \cdot 0001$
Mate status	1	$5 \cdot 583\text{E-}5$	$0 \cdot 002$	$0 \cdot 9662$
Interaction	2	$0 \cdot 024$	$0 \cdot 791$	$0 \cdot 4588$
Residual	54	$0 \cdot 031$		

Choice of smaller hosts

The number of eggs laid on each host type was estimated by the number of emerging wasp offspring divided by the estimate of wasp offspring survival rate for each host type (see above). To compare the host choice decision of inseminated and virgin female wasps, the proportion of eggs laid on small hosts (12- or 13-day-old hosts) was compared with eggs laid on all hosts. The proportions were normalized by an arc-sine square root transformation.

Interaction between the two factors was significant (Table 2). For the inseminated wasps, the proportion of eggs laid on the smaller hosts increased in choice type Ch13/17 compared with choice type Ch12/17. On the other hand, for virgin wasps, the proportion of eggs laid on the smaller hosts did not differ in both choice types (Fig. 1).

Number of eggs laid in the first 6 days

The numbers of eggs laid per 24-h period were compared. Data from the first 6 days of parasitization were used in each experiment in order to eliminate the effects of variation in longevity, egg deficiency and other factors correlated with the stochasticity of lifetime events. A two-way ANOVA showed no significant effects of interaction and wasp status on the number of eggs laid in the period, but a significant effect of choice type (Table 3).

Table 2. ANOVA table of the effects of mating status and host age on the proportion of *D. basalis* eggs laid on smaller hosts

	df	MS	*F*	*P*
Mate status	1	0·992	20·956	0·0002
Interaction	1	0·251	5·704	0·0275
Residual	19	0·044		
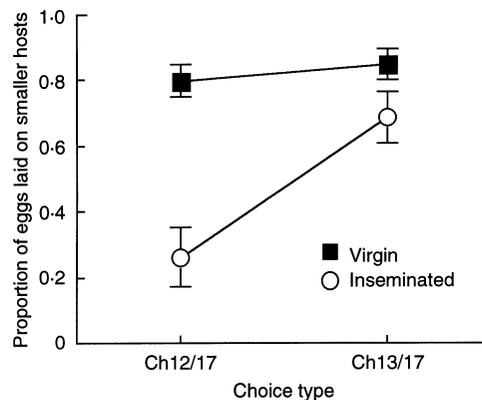
Fig. 1. Proportion of eggs laid by *D. basalis* on small (12- or 13-day-old) hosts. Vertical bars indicate standard errors.

Table 3. ANOVA table of the effects of mating status and host age on the number of *D. basalis* eggs laid in the first 6 days of oviposition

	df	MS	*F*	*P*
Mate status	1	0·247	0·001	0·9780
Interaction	1	249·318	0·789	0·3854
Residual	19	315·792		

Table 4. ANOVA table of the effects of mating status and host age on longevity of *D. basalis*

	df	MS	*F*	*P*
Mate status	1	38·629	6·661	0·0439
Interaction	1	32·914	3·971	0·0608
Residual	19	8·288		

Longevity of mothers

Only wasp status significantly affected wasp longevity (see Table 4). Virgin females lived longer (14·545 ± 4·677 SD days) than inseminated ones (11·667 ± 2·060 SD days).

Lifetime fecundity

The effect of wasp status on the number of eggs laid through the wasp's lifetime was statistically significant (Table 5). Virgin females produced more eggs (277·630 ± 101·789 SD) than inseminated ones (203 ± 70·491 SD).

Discussion

The virgin females differed in host selection decisions from inseminated females and consequently lived longer, laying many eggs during the choice experiments. The consequences for life-history parameters, fecundity and longevity, are all caused by the behavioural aspects of host choice. Although mating cost has been reported in *Drosophila melanogaster* (Chapman *et al.* 1995), insemination status did not appear to be a direct factor influencing longevity in *D. basalis*.

Host mass is positively correlated with the mass of wasp offspring (see Nishimura 1993: Figure 2). It is broadly accepted that a relative change in fitness with change in body size is greater for females (Charnov 1979; Heinz 1991; King 1992, 1993). In addition, inseminated females usually lay a greater proportion of female eggs on large hosts (Charnov 1979; Werren 1984), which is the case in *D. basalis* (Nishimura 1993).

If the female wasp utilizes both small and large hosts, laying many female eggs on large hosts and many male eggs on small hosts is an efficient decision (Werren & Simbolotti 1989). Previous studies have

Table 5. ANOVA table of analysis of the effects of mating status and host age on lifetime total of *D. basalis* eggs

	df	MS	F	P
Mate status	1	27 803·709	5·040	0·0369
Interaction	1	15·931	0·003	0·9577
Residual	19	5 516·693		

rarely considered utilization patterns of small and large hosts with respect to offspring sex allocation. However, if different costs exist in laying eggs on large and small hosts, not only offspring fitness but also oviposition cost for the female wasps should be considered when explaining host utilization patterns. Oviposition cost and offspring fitness trade-off would determine the host choice pattern.

Generally, oviposition cost would be quantified by energy expenditure and/or time to oviposit an egg (including searching and handling the host) (Stephens & Krebs 1986). When there exists a reproduction and survival trade-off, three behavioural options can be considered: rest (non-reproductive behaviour), choosing a small host and choosing a large host. In this experiment it was difficult to tell whether any limitations occurred in time and/or energy cost. Even though oviposition costs in neither energy nor time could be quantified in the experiment, the wasps' searching ability was excellent and oviposition time short (within 10 min, personal observation). Female *D. basalis* lay about 15 eggs in 24 h under the most suitable conditions (high density of large hosts: minimum search time) and the same number of eggs were laid in similar experimental settings (see Nishimura 1993). Accordingly, oviposition costs should be considered as an energy cost, rather than a time cost, and the experiment well demonstrates the trade-off between difficulty of oviposition and offspring rearing conditions.

Within the inseminated wasps, choice changed with host choice type. This may reflect a change in energy investment for laying eggs and offspring fitness balance between the two choice situations (see Fig. 1). In Ch12/17 female wasps chose 17-day-old hosts more often. The wasps lay more fertilized eggs on large hosts and more unfertilized eggs on small hosts in a similar choice situation, according to Nishimura (1993). Choosing large hosts and incurring greater costs might pay off by laying fertilized eggs on large hosts. In Ch13/17 smaller hosts (13-day-old hosts) were chosen more often. The fitness offspring emerging from 13-day-old hosts would be relatively high compared with that offspring emerging from 12-day-old hosts, whereas the energy cost of laying eggs on aggregated 13-day-old hosts might be relatively low compared with that for sparse 17-day-old hosts. Therefore, the wasps chose to pay a lesser cost.

On the other hand, fitness can be acquired only through male offspring from constrained (virgin) female wasps. If the relative fitness of male offspring emerging from smaller hosts is greater than that of female offspring arising under similar conditions from inseminated females for the virgin female, the energy saved by laying eggs on smaller hosts may be a good strategy in order to lay as many eggs as possible. Virgin females can conserve energy by choosing small hosts; the unfertilized eggs not being subject to any greater disadvantage than fertilized ones laid on small hosts. In both experiments the virgin wasps chose more small aggregate hosts than large sparsely distributed hosts.

The search efficiency over a 24-h period for each host type can be inferred from the number of eggs laid (emergent offspring). Search efficiencies were similar between the inseminated and virgin females within the same choice type experiments (see Table 3, *P*-value of Mate status), indicating similar host searching abilities.

The virgin wasps chose a greater proportion of small hosts and laid more eggs in their lifetime. On the other hand, inseminated females, producing both male and female offspring, incurred greater costs by laying fertilized eggs on large hosts; consequently, their life expectancy was shortened and fecundity decreased compared with virgin females.

The trade-off between reproduction and survival has often been discussed (Reznick 1985; van Noordwijk & de Jong 1986; reviewed in Stearns 1992; Bell & Koufopanou 1986; Roff 1992; Stearns 1992). The results presented in this paper provide a behavioural basis for such a trade-off under a certain conditions. By comparing host utilization patterns and the fitness consequences for virgin and inseminated females it can be concluded that the wasps change their behaviour effectively according to their inseminated condition.

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