

BROOD SIZE AND SEX RATIO REGULATION IN THE  
PARASITIC WASP *NASONIA VITRIPENNIS* (WALKER)  
(HYMENOPTERA: PTEROMALIDAE)

by

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SUMMARY

The parasitic wasp *Nasonia vitripennis* varies its brood size and sex ratio dependent upon whether it is the first or second wasp to parasitize a fleshly (*Sarcophaga bullata*) pupa. Some of the proximate causes of this variability were investigated. In particular, research was conducted to investigate (1) effect of time between parasitization upon the probability of superparasitism, brood size and sex ratio of the superparasite, (2) whether the sex and number of larvae in host affects the oviposition behavior of the superparasite and (3) whether there is a temporal sequence in laying sons versus daughters during parasitization.

It was found that the probability of superparasitism decreased, average brood size of the superparasite decreased, and proportion of sons increased with increasing time since the previous parasitization. A closer examination revealed that with a 3-24 hour delay between attacks, there is no significant relationship between brood size and sex ratio of the superparasite or between brood size and sex ratio of the superparasite or between brood size of the first wasp and brood size of the superparasite. However, by a 48 hour delay, there was a significant negative relationship between brood size and proportion sons of the superparasite. In addition, a significant **negative relationship between the brood size of the first and second wasp developed at a 48 hour delay, suggesting that the superparasite is able to detect large differences** in first wasp brood size. The presence of feeding larvae in a host, at a 48 hour delay, significantly decreases the probability of superparasitism, but does not reduce the brood size of those wasps which do superparasitize.

In *N. vitripennis*, a first wasp to parasitize a host does not lay sons first in the oviposition sequence. This contrasts to the pattern observed in certain other species. Data on the superparasite are less clear, but suggest that sons may be laid early in the bout.

Based on the data, it is hypothesized that *N. vitripennis* has two sex ratio patterns. In the "primary parasite pattern" a strongly daughter biased sex ratio is produced and sex ratio is independent of brood size. In the "superparasite pattern" sex ratio is strongly influenced by brood size. Typically 100 To sons are produced when few eggs are laid, but the proportion of daughters increases with increasing number of eggs laid into the host.

Results are discussed in relation to ecological theory and in particular to models of sex allocation under superparasitism in parasitic wasps.

' "The views of the author do not purport to reflect the position of the Department of the Army or the Department of Defense," (para 4-3, AR 360-5).

## INTRODUCTION

Sex ratios are highly variable among the parasitic hymenoptera (CLAUSEN, 1940; FLANDERS, 1956) and the adaptive significance and proximate causes of this variability is currently the focus of intensive study. Two effects play significant roles in shaping the adaptive sex ratio among parasitic wasps. These are local mate competition (HAMILTON, 1967) and host quality (CHARNOV, 1979). Local mate competition occurs in small local populations in which mating occurs among the progeny of a few parents, with daughters then dispersing into the larger population before forming new denies for the next generation. Hamilton showed that such population structures favor the evolution of daughter biased sex ratios. Local mate competition is expected in parasitic arthropod species in which one or a few parents lay eggs in a host (or a patch of hosts), with mating occurring among the progeny from that host (or patch) (HAMILTON, 1967, 1978).

Host quality influences sex ratio selection when it has a differential effect upon the relative fitness (survival and fecundity) of males and females (CHARNOV, 1979). For example, a poor host may result in reduced size of male and female offspring. If the fitness of females is more adversely influenced by small size than the fitness of males, then selection will favor producing a greater proportion of sons in poor hosts. Local mate competition and host quality effects are likely to co-occur in many wasp species, and will be influenced by the brood size strategy of the wasp (WERREN, 1984).

The parasitic wasp, *Nasonia vitripennis*, is a useful organism for studying local mate competition and host quality effects. Of special interest is the "superparasitism" behavior of this species. The first wasp to parasitize a host (fly pupa) produces a strongly daughter-biased sex ratio, but when a second wasp "superparasitizes" the same host, she (1) reduces her brood size and (2) produces a more male biased sex ratio (WYLIE, 1965, 1966; HOLMES, 1972; WERREN, 1980a, b).

Superparasitism provides an excellent test of sex ratio theory because the optimal sex ratio of the first and second wasp can differ dramatically. WERREN (1980a) developed a local mate competition model for superparasitism. It assumes random mating between the two broods and high offspring survival in superparasitized hosts. The model shows that three factors—the brood size of the first wasp, the sex ratio of the first wasp, and the brood size of the second wasp— influence the optimal sex ratio of the second parasite. In general, if the superparasite brood size is small relative to the primary parasite brood size, the superparasite should produce 100 percent sons, but as her relative brood size increases, she should produce a more daughter

biased sex ratio. In contrast, the optimal sex ratio of the first parasite depends upon the probability of superparasitism, and should fall somewhere between 0 and 25 percent sons. SUZUKI & IWASA (1980) considered the additional impact of host quality upon the optimal strategies.

Experiments have shown that the wasps do generally vary sex ratio as predicted by the model (WERREN, 1980a). However, considerable variability was found in the brood size of the superparasite. This variability suggested that the wasp was adjusting the number of eggs she laid into a host as a response to certain environmental factors.

The purpose of the research presented here is to investigate some of the environmental factors involved in brood size and sex ratio regulation in *N. vitripennis*. In particular, research was conducted to investigate (1) effect of time between parasitization upon the probability of superparasitization, brood size and sex ratio of the superparasite, (2) whether the sex and number of larvae in a host affects the oviposition behavior of the superparasite and (3) whether there is a temporal sequence in laying sons versus daughters during parasitization. Results from these experiments are related to the expectations of theory.

#### BIOLOGY OF *NASONIA*

*N. vitripennis* is a small (3 mm) chalcidoid wasp which parasitizes many species of cyclorrhaphous fly pupae (WHITING, 1967), especially those of the blowflies (Calliphoridae, Sarcophagidae). The wasp has been studied genetically and mutants have been characterized (SAUL et al., 1965).

Upon encountering a host, a female wasp examines it, drills through the pupal wall with her ovipositor and then stings the pupa. "Venoms" are injected which kill the host and retard decay of the host body (BEARD, 1964; RATCLIFFE & KING, 1967). After stinging, the wasp deposits a batch of eggs within the puparium. She then drinks host fluid from the sting site and uses that nutrition for the maturation of new eggs. This pattern may be repeated several times upon the same host before the wasp moves on in search of other pupae (EDWARDS, 1954). At 25 °C, offspring develop into adults within 14 days. Wasp pupae eclose within the host and remain there for approximately 24 hours before emerging. Mating occurs around the host after emergence and females typically mate only once (VAN DEN ASSEM & FEUTH-DE BRUIJN, 1977). The males are flightless (wings are vestigial) and shortlived (KING & HOPKINS, 1963).

As in other hymenoptera, ploidy determines sex: unfertilized (haploid) eggs develop into males and fertilized (diploid) eggs develop

into females. This provides the wasp a potential for controlling the sex ratio amongst her offspring (FLANDERS, 1956; KING, 1962a, b). Previous studies have demonstrated that *N. vitripennis* does exercise some control over sex ratio. (WYLIE, 1966; WERREN, 1983).

## METHODS

### *"Subheading Nuclea: Add in Proof"*

Two genetic strains of *N. vitripennis* were used in experiments; a "wild-type" (+) stock from Carolina Biological Supply Company and a "scarlet-eye" (sc) mutant of the 'R' locus (SAUL *et al.*, 1965). Both are highly viable. The two genotypes were used to distinguish between offspring of the primary and superparasite broods. Stocks were maintained by placing 15 inseminated females into a vial with 10 cc of hosts. *Sarcophaga bullata* pupae, which were provided twice weekly by Carolina Biological Supply Company, were used as hosts. They were refrigerated at 7°C upon arrival. The pupae were warmed to room temperature for two hours before experimental use. Hosts remain viable under refrigeration for over one month, but only hosts which had been in the refrigerator for less than 1 week were used in experiments.

Progeny which had emerged within the previous 36 hours from the stock cultures were used for experimental purposes. Since drinking of host fluid is needed for wasp egg production (WYLIE, 1966; WHITING, 1967), the wasps were first exposed to a routine of hosts and honey. Unless otherwise indicated, each wasp was initially provided hosts (20 wasps with 20 hosts) for 24 hours and then given honey singly for 18 hours in a 12 x 75 mm test-tube plugged with cotton. Each wasp was then provided a single host for a period of time dictated by the particular experiment. All experiments were conducted at  $24 \pm 1$  °C under constant light.

Hosts with more than two diapausing larvae were excluded from data analysis. The t test was used to statistically compare means, and sex ratios (proportion sons) were analyzed after an arcsin square root transformation to approximate normality (SGKAL & ROLF, 1969). A simple linear regression is used to compare relationships between variables.

Previous experiments conducted under similar circumstances have shown that egg to adult mortality of wasps is low when *S. bullata* hosts are utilized (WERREN, 1980b). Therefore, unless indicated otherwise, adult emergencies are used as an index of the number of eggs laid into a host.

The first three experiments investigate the effect of time between attacks of the first and second wasp upon the behavior of the second wasp. The other experiments determine whether the superparasite can detect the brood size or the sex ratio of the first wasp, and document the temporal pattern of egg laying by the primary parasite.

### *Effect of Time Between Attacks*

The effects of time between first and second parasitization were investigated in experiments A, B and C. The purpose of experiment A was to determine whether the second wasp could detect previous parasitization soon after onset of the first parasitization. The experimental design was as follows: wasps were provisioned in the usual fashion, and then isolated singly in test tubes. Individual hosts were exposed to a wasp for 3 hours. The host was then removed and given to a second wasp for 3 hours. Alternate genotypes were used for the first and second wasp. In one case scarlet-eye was

the first parasite and in the other wild-type was the first. In addition, a no superparasitism control was conducted for each genotype.

Experiments B & C explored the effects of increasing time delays between the two parasitizations. Experiment B used 3, 24, 48 and 72 hour delays and Experiment C used 24, 48 and 72 hour delays. In these experiments scarlet-eye females were presented hosts first and wild-type females were used as the second wasps. All wasps were pretreated in the usual fashion.

#### *Effect of Larval Number and Sex*

Experiment D was conducted to test the hypothesis that a second wasp can detect the number of larvae in a host, and that she adjusts her brood size accordingly. The experimental design utilized irradiated males. The fertilized eggs produced by females mated to irradiated males (5000 roentgens) typically die prior to hatching into larvae. Unfertilized (male) eggs hatch normally and develop into adults. Females mated to irradiated~ males lay the same number of eggs as do normal females and presumably deposit the same quantity of venom. But 48 hours later hosts from the irradiated group contain a few feeding larvae and many inviable eggs while the control contains many feeding larvae. For the experiment, scarlet-eye females were mated with either irradiated or unirradiated males, provisioned singly with a host for 24 hours and then given honey for an additional 24 hours. They were then provided a host singly for 6 hours. Forty-eight hours after onset of the first parasitization, normally mated wild-type **females were exposed singly to the hosts for 6 hours to allow superparasitism.** A second unirradiated control was run in which hosts were opened and the number of superparasite eggs were counted, for an estimate of mortality among the second brood.

Experiment E was conducted to determine the effect of the sex ratio of the first brood upon the second wasp. Sex ratio theory predicts that a superparasitizing wasp should adjust her sex ratio in response to the sex ratio of the first wasp so as to maximize reproductive returns. This was tested by using virgin females (who produce all-male broods) as primary parasites. Theory predicts that in the presence of an all-male brood, the second wasp should produce a more daughter biased sex ratio.

#### *Sequence of Laying Sons and Daughters*

These experiments (1) test whether the 1st and 2nd wasps lay one sex before the other during oviposition and (2) demonstrate the temporal pattern of egg laying upon the host. In experiment F, individual wasps were allowed to oviposit on a host for 2, 4, 6 or 8 hours to determine the effect of time on the host upon the sex ratio. Experiment G focused further upon the sex ratio shift. Females were provisioned singly with a host for 24 hours and then were depleted of mature eggs by providing them with two successive hosts for 4 hours each. Each was then given a host for either 2 or 12 hours. If one sex offspring is laid before the other, then sex ratio among broods should change with time upon the host.

A final experiment (H) examined the sequence of laying sons versus daughters by wasps encountering previously parasitized hosts. Two replicates of the experiment were performed. In each, the first (scarlet-eye) wasp was mass provisioned in **the usual fashion for 24 hours and then given a host for 12 hours. Twenty-four hours after** onset of the first parasitization, the host was presented to a wild-type female for 3, 6 or 12 hours. The second wasp had been similarly provisioned.

## RESULTS

*Effect of Time Between Attacks*

The purpose of experiment A was to determine whether the second wasp could detect previous parasitization soon after onset of the first parasitization. The data presented in table 1, show that a wasp can detect previous parasitization even when it occurs only three hours previously. The wasp responded by producing a greater proportion of sons. The superparasite's sex ratio was greater than the control for both genotypes (wild-type,  $p < .01$ ; scarlet-eye,  $p < .0001$ ). The sex ratio shift cannot possibly be explained by increased mortality of daughters, since not only the proportion, but the absolute number of males increased (wild-type  $p < .05$ ; scarlet-eye,  $p < .001$ ). For both genotypes, the brood size of the superparasite was also reduced, but not significantly. This lack of significance could be due to small sample size.

TABLE I

Results from Experiment A which show the mean  $\pm$  S.E. number of sons, percent sons, and brood size of the primary and **superparasite when the** second attack occurs 3 hours after the first attack. For each genotype, the superparasite is compared to a no superparasitism control and significant differences are indicated (\* =  $p < .05$ ; \*\* =  $p < .01$ ; \*\*\* =  $p < .001$ ). These significance symbols will be used for all subsequent tables.

	Superparasitism		Single parasitism control
	1st Wasp	2nd Wasp	
Wild type			
Number sons	1.3 $\pm$ 0.4	3.0 $\pm$ 0.7*	1.4 $\pm$ 0.3
Percent sons	<del>6.0</del> $\pm$ 1.8	21.8 $\pm$ 4.2**	<del>6.4</del> $\pm$ 1.0
Brood size	17.6 $\pm$ 2.5	16.6 $\pm$ 4.1	19.7 $\pm$ 2.4
Sample size	12	17	14
Scarlet eye			
Number sons	2.0 $\pm$ 0.4	5.6 $\pm$ 1.0***	1.5 $\pm$ 0.3
Percent sons	10.6 $\pm$ 2.0	38.7 $\pm$ 6.8**	<del>7.4</del> $\pm$ 1.4
Brood size	17.9 $\pm$ 2.1	14.2 $\pm$ 1.5	18.7 $\pm$ 2.1
Sample size	17	12	15

Experiments B & C explored the effects of increasing time delays between the two parasitizations. To control for genotype, only wild-type wasps were used in the no superparasitism control and as superparasites in the time delay groups. The effects of time between attacks are summarized in table 11. The data reveal that as time since previous parasitization increases a wasp (1) is less likely to superparasitize a

host, (2) produces a smaller brood size if she does superparasitize and (3) produces an increasing proportion of sons.

TABLE II

The effect of time between attacks upon probability of superparasitism, superparasite brood size and superparasite sex ratio, is presented. Data combine results from experiments A, B, & C plus a no superparasitism control.

	Control	Time between attacks			
		3	24	48	72
Proportion					
Superparasitized	—	.93	.89	.56***	.33*
(N)	—	(41)	(43)	(78)	(63)
Broodsize	19.3 ± 0.9	15.5 ± 1.6*	12.8 ± 1.1	8.3 ± 1.0**	8.2 ± 1.7
Sex ratio					
(arc sin,					
prop. sons)	16.6 ± 1.7	24.8 ± 3.5*	31.4 ± 3.5	56.9 ± 4.3***	32.2 ± 6.1**
(N)	(51)	(38)	(38)	(44)	(21)

\* *etc.*, see TABLE I.

The probability of superparasitism is initially high (around 90%), but drops with a delay of 24 and 48 hours. This pattern is similar to that observed by KING & RAFAI (1970). There is an immediate although small drop in the parasites brood size with a three hour delay. As pointed out, this reduction in brood size is unlikely to be due to mortality because of the high survivorship of wasps on these hosts (WERREN, 1980b). There is little change in the brood size with a 3 to 24 hour delay, but a clear decrease with a 48 hour delay between parasitizations.

The pattern repeats itself with sex ratio. There is a significant increase in the proportion of sons a wasp produces upon a host, even if previously parasitization occurred only 3 hours previously. The sex ratio continues to increase at 24 hour delay, but there is a large significant increase with a 48 hour delay. In the mean sex ratio, a significant decline is then observed with a 72 hour delay. The decline is believed to be an artifact of the experimental design, as will be described later. The general pattern fits the expectations of sex ratio theory (WERREN, 1980a). As the brood size of the superparasite decreases, her sex ratio increases.

A closer analysis reveals some of the dynamics in brood size and sex ratio regulation. Figure 1 plots the relationship between sex ratio and brood size of the superparasite over the time course. In the no superparasitism control, sex ratio is strongly female biased and there is no

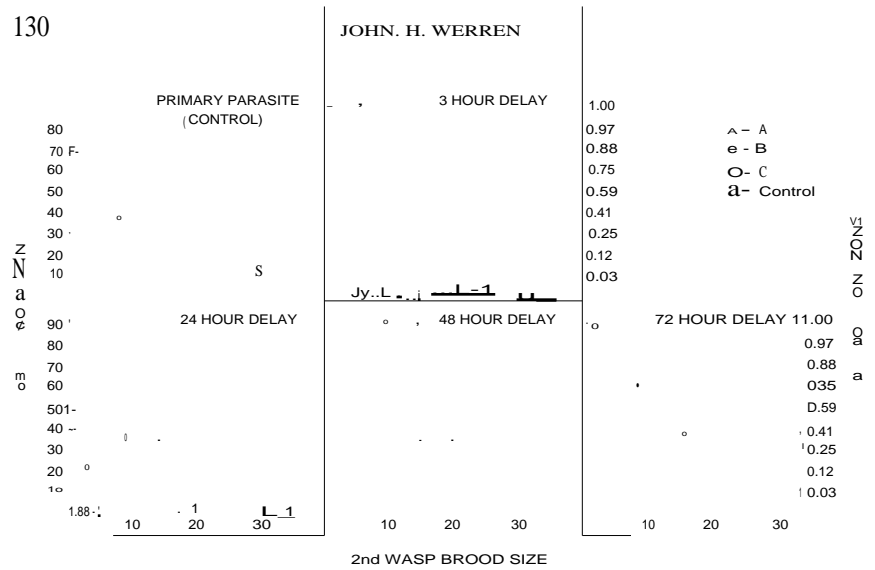


Fig. 1. The sex ratio of the superparasite is plotted with respect to superparasite brood sizes from experiments A (•), B (○) and C (○) for 3, 24, 48 and 72 hour delays between parasitizations. The primary parasite control is taken from A, C and a separately run experiment (○).

relationship between sex ratio and brood size of the wasp ( $r = -.2$ ,  $n = 52$ ). When the wasp superparasitizes a host which was parasitized 3 hours previously, she increases her sex ratio, however there is again no relationship between brood size and sex ratio ( $r = .07$   $p > .6$ ,  $n = 38$ ). All of the second wasps may not have detected previous parasitism, since many of the points overlap with the control. After a 24 hour delay, a relationship still does not occur between brood size and sex ratio ( $r = .15$   $p < .3$ ,  $n = 38$ ), although the average sex ratio has increased further. However by a 48 hour delay there is a dramatic change in the pattern. A strong negative correlation is found between brood size and sex ratio of the superparasite ( $r = -.51$ ,  $p < .001$ ,  $n = 44$ ). A 72 hour delays shows a similar (although) nonsignificant negative correlation ( $r = -.25$ ,  $p < .2$ ,  $n = 21$ ).

Interesting similarities are found when comparing brood sizes of the first and second wasps, as shown in figure 2 and table III. Table III presents the mean and standard error of the brood sizes for experiment B & C. The correlation coefficient of a simple linear regression between first and second brood size in superparasitized hosts is shown. Note that the correlation excludes those hosts which were not superparasitized. The mean and standard error of first brood size for those hosts which were not superparasitized is also presented.



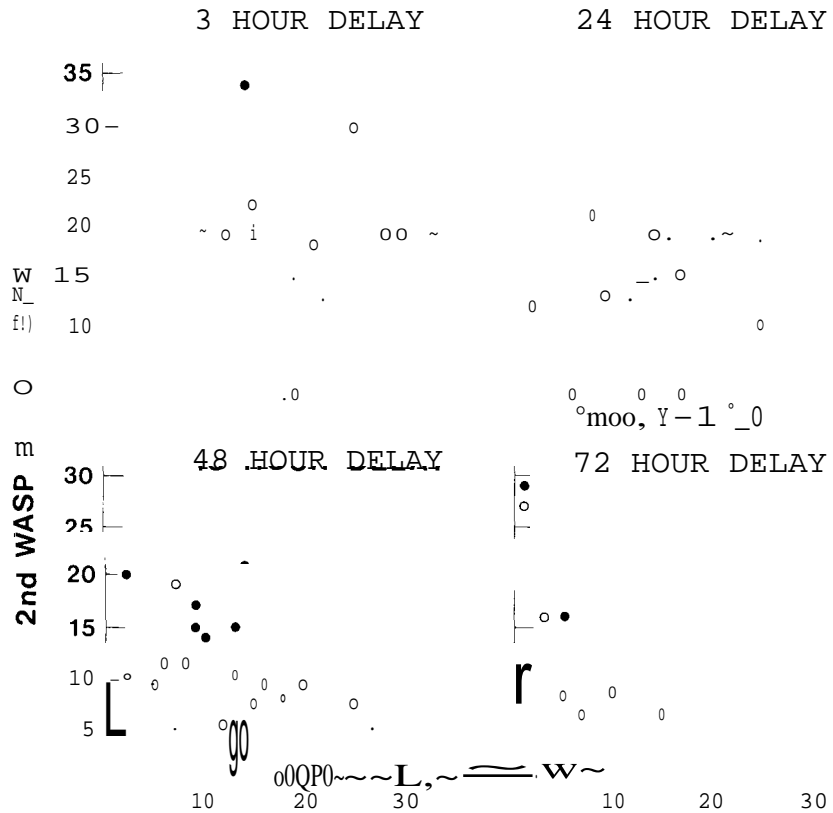


Fig. 2. Superparasite brood size is plotted with respect to primary brood size from Experiments A (A), B (•) and C (°) for 3, 24, 48 and 72 hour delays between parasitizations. Correlation coefficients from these experiments are shown in table III.

From figure 2 and table III, it is clear that there was a great deal of variance in brood size. Many fewer offspring were laid into hosts in these experiments than when wasps are allowed free access to hosts (WERREN, 1980b). This occurred because the wasps were exposed to the hosts for only three hours and then removed, often times before a normal parasitization was complete.

With a three hour delay between attacks, there is no significant correlation between first and second brood sizes. Similarly, at 24 hours there is no relationship. However, as with the sex ratio data, a dramatic change occurs between 24 and 48 hours. With 48 hours delay, a negative correlation between first and second brood size has

TABLE III

The mean  $\pm$  S. E. brood sizes of primary and superparasite are shown for Experiments B and C. Data are separated into superparasitized and not superparasitized hosts. The correlation coefficient of a simple linear regression between first and second brood size in superparasitized hosts (excluding unsuperparasitized hosts) is also shown.

Exp.	Delay	(N)	Host superparasitized			Host not superparasitized	
			2nd Brood	1st Brood	R	1st Brood	(N)
B	3 hr	(21)	14.9 $\pm$ 2.0	16.3 $\pm$ 1.5	.08	13.0 $\pm$ 3.4	(3)
	24 hr	(21)	15.8 $\pm$ 1.1	16.4 $\pm$ 1.4	.01	14.5 $\pm$ 1.5	(2)
	48 hr	(23)	8.7 $\pm$ 1.3	13.8 $\pm$ 1.5	-.30	19.9 $\pm$ 1.9*	(19)
	72 hr	(11)	<u>7.6</u> $\pm$ 2.5	12.0 $\pm$ 2.3	-.43	19.1 $\pm$ 1.6*	(29)
C	Control	(17)	17.7 $\pm$ 1.4				
	24 hr	(17)	8.2 $\pm$ 1.5	12.0 $\pm$ 1.5	.03	13.7 $\pm$ 5.9	(3)
	48 hr	(21)	<u>6.0</u> $\pm$ 1.8	14.3 $\pm$ 1.0	-.41**	17.1 $\pm$ 1.7	(15)
	72 hr	(10)	<u>8.0</u> $\pm$ 2.5	8.1 $\pm$ 1.6	-.73**	14.4 $\pm$ 2.0*	(13)

\* etc., see TABLE I.

developed. There was a significant negative relationship for experiment C and a nearly significant one for experiment B.

This result indicates that the superparasite can "detect" the brood size of the first wasp and that she increases her brood size accordingly. Primary brood size in singly parasitized hosts was significantly greater than that in superparasitized hosts (table 111), which further supports the interpretation that the second wasp is sensitive to at least large differences in first brood size 48 hours after the initial attack.

Clearly, there are changes which occur within the host between 24 and 48 hours after initial parasitization which influence how a wasp responds to the host. It is pertinent to note that at 25 ° C eggs hatch into larvae around 36 hours after oviposition. With a 48 hour delay between attacks the superparasite appears capable of detecting large differences in the number of first wasp offspring in the host (hence the negative correlation between first and second brood size). There are at least two hypotheses to explain this; (1) the larvae produce some substance correlated with their number (e.g. CO<sub>2</sub>, other waste products) which can be detected by the superparasite or (2) the amount of "venoms" or other substances injected into the host during stinging by the parent is correlated with the number of eggs she deposits in the host. In this case, either the superparasite does not respond to the cue before the 48 hours, or the relative level of the substance is not detectable before then.

*Effect of Larval Number and Sex*

Experiment D was conducted to test the hypothesis that a second wasp can detect the number of larvae in a host, and that she adjusts her brood size accordingly. As described, the experimental design utilized irradiated males. The fertilized eggs produced by females mated to irradiated males typically die prior to hatching into larvae. Forty-eight hours after the initial parasitization, hosts from the irradiated group contain a few feeding larvae and many inviable eggs while the control contains many feeding larvae.

Table IV shows the response of the second wasp to these two circumstances. Egg counts were made of a second control to verify that the brood size and proportion of superparasitized hosts in the control were not due to increased mortality in the second brood. Data show that the second wasp was significantly less likely to superparasitize hosts which contained many feeding larvae. However, if a wasp did superparasitize a host, the number of larvae had no significant effect upon the brood size she produced. The second brood size (combined egg and adult count  $6.7 \pm 0.9$  SE,  $n = 21$ ) was not significantly different from in the irradiated group ( $8.7 \pm 1.4$  SE,  $n = 26$ ). Therefore, the number of larvae in a host does not explain the negative relationship between first and second brood size observed at 48 hours, but does play a role in the inhibition of superparasitism. Apparently, several host cues affect the response of a wasp to a previously parasitized host. The presence of feeding larvae affects the probability of superparasitism, but other cues, possibly changes associated with the amount of venom injected into a host, regulates the egg number deposited.

TABLE IV

Results are presented from an experiment to determine the influence of larvae in a host upon the behavior of the superparasite. The probability of superparasitism, and mean  $\pm$  S.E. brood sizes of the first and second wasp are shown for superparasitized and not superparasitized hosts. In the control, first females were mated to nonirradiated males. For control egg, hosts were opened and the egg number of the second brood was counted. In control adult, offspring were allowed to emerge. In irradiated mate, offspring were allowed to emerge. The first females had been mated to irradiated males, which caused lethality of most of the eggs.

	<i>Probability of superparasitization</i>	<i>(N)</i>	<i>Host superparasitized</i>		<i>(N)</i>	<i>Host not</i>	<i>(N)</i>
			<i>2nd Brood</i>	<i>1st Brood</i>		<i>1st Brood</i>	
Control egg	.282	(33)	7.4 $\pm$ 1.4	—	(11)	—	
Control adult	.208	(48)	5.9 $\pm$ 1.1	28.5 $\pm$ 2.1	(10)	37.8 $\pm$ 1.6**	(38)
IRR. mate	.578	(46)	8.7 $\pm$ 1.4	5.6 $\pm$ 0.5	(26)	6.4 $\pm$ 0.7	(20)

see TABLE I.

Another experiment was conducted to determine the effect of the sex-ratio of the first brood upon the second wasp. Theory predicts that a superparasitizing wasp should adjust her sex-ratio in response to the sex-ratio of the first wasp so as to maximize reproductive returns. Virgin females (who produce all-male broods) were utilized as primary parasites to investigate this. Theory predicts that in the presence of an all-male brood, the second wasp should produce a more daughter biased sex ratio.

Results show that the sex ratio and brood size of the second wasp was not significantly influenced by whether the first wasp was a virgin (brood size  $8.1 \pm 1.4$  SE,  $N = 20$  versus  $8.0 \pm 1.1$  SE,  $n = 12$ ; arc sine sex ratio  $30.1 \pm 7.0$  SE versus  $20.6 \pm 4.8$  SE,  $p > .30$ ).

#### *Sequence of Laying Sons and Daughters*

A further topic of investigation was the sequence of laying sons versus daughters. In some parasitic wasps, there is a definite sequence to the laying of sons and daughters, with sons being laid first in the oviposition sequence (GREEN *et al.*, 1982; WAAGE & LANE, 1984). Two sets of experiments were conducted. One investigates the sequence in the first wasp and the other investigate the sequence in the second wasp on a host.

Table V shows the oviposition pattern of primary parasites. In experiment F, individual wasps were allowed to oviposit on a host for 2 to 8 hours. Of the 1 hour group, 90% of the hosts were parasitized and

TABLE V

The effect of time on a host upon the mean  $\pm$  S.E. brood size, proportion all-female broods and sex ratio is presented. In experiment F, the wasps were on the hosts for 1, 2, 4, or 8 hours. Experiment G, the females were first depleted of eggs and then put on a host for 2 or 12 hours. Sample size for sex ratios are the pooled number of progeny per group.

	<i>Brood size</i>	<i>Proportion all-female broods</i>	<i>(N)</i>	<i>Percent males</i>	<i>(N)</i>
<i>Experiment F</i>					
1 hr	12.5+ 1.7	.33	(20)	4.9	(250)
2 hr	17.4+ 1.5*	.10*	(40)	7.4	(696)
4 hr	24.6+ 1.9**	.04	(26)	13.9***	(640)
8 hr	27.5 $\pm$ 1.8**	.03	(30)	11.6	(825)
<i>Experiment G</i>					
2 hr	5.6 $\pm$ 0.7	.71	(17)	5.3	(95)
12 hr	21.6 $\pm$ 1.7**	0**	(17)	20.3**	(344)

\* etc., see TABLE 1.

by 8 hours all 100% were parasitized. Brood size increased with increasing time on the host, demonstrating that successive batches of eggs are laid (EDWARDS, 1954). The mean sex ratio increased from 4.9% sons to 11.6% sons. Therefore, a higher proportion of sons were laid later in the oviposition series. This experiment does not reveal, however, whether sons are laid early or later in a single oviposition bout.

Experiment G focused upon the sex ratio sequence in a single oviposition bout. Females were depleted of mature eggs by providing them with two successive hosts and then each was given a host for either 2 or 12 hours. Results from table 11 show that even when the wasp had few mature eggs, she did not wait but immediately deposited them within a host. Approximately 5% sons were laid in the first two hours in both experiments F and G, even though many more eggs were laid in that time in experiment F. The number of eggs laid in the experiment G 2 hours group ranged from 1 to 10. The high proportion of all-daughter broods shows that sons are not laid first in the oviposition bout. This contrasts to the system in *Trichogramma*, where sons have a much higher probability of being produced early in an oviposition series (WAAGE & LANE, 1984).

A final experiment examined the sequence of laying sons versus daughters by wasps encountering previously parasitized hosts (Experiment H). The first (scarlet-eye) wasp was given a host for 12 hours. Twenty-four hours after onset of the first parasitization, the host was presented to a wild-type female for 3, 6, or 12 hours.

In both replicates, there was ample parasitization by the first wasp (brood size  $26.4 \pm 10.0$  SD (N = 66) for replicate 11). This contrasts to the lower level of parasitization when the first wasps were on the hosts for only 3 hours. The oviposition pattern of the second wasp differs somewhat from that of the first wasp (table VI). Both replicates show a

TABLE VI

The effect of time on a previously parasitized host upon the proportion of hosts superparasitized, brood size and sex ratio of a superparasite, is presented. The sample size for sex ratios represents the number of hosts, with sex ratio calculations being percent males per host. Brood size calculations exclude the unsuperparasitized hosts.

		<i>Broodsize ± S. D.</i>		<i>Percent male ± S.D.</i>	<i>Sample size</i>	<i>Proportion superparasitized</i>	<i>Sample size</i>
I	3 hr	<u>3.6±</u> 2.5		<u>65±</u> 42	8	.36	22
	6 hr	<u>3.5±</u> 3.3		<u>78±</u> 34	13	.62	21
	12 hr	<u>5.5±</u> 5.3		41 + 39	16	.70	23
11	3 hr	<u>3.6±</u> 2.4		<u>70±</u> 34	11	.52	21
	6 hr	<u>7.0±</u> 6.4		<u>59±</u> 37	17	.77	22
	12 hr	15.0+ 15.0		41 + 28	16	.89	18

gradual increase in the percent superparasitism and brood size, and a gradual decrease in proportion of sons with time on the host. For example, in replicate II approximately 50% of the hosts are superparasitized in the first 3 hours. There is a non-significant increase by 6 hours, and a significant increase by 12 hours (3 hours, prop. superparasitized = .52 versus 12 hours, prop. superparasitized = .89,  $p < .01$ ).

Although the sex ratio does decrease with time on the host, it does not appear to be due to a sequence of laying sons first and then daughters later in a continuous oviposition series. Rather some wasps appear to oviposit early and produce a small brood size and high sex ratio, whereas others oviposit later and produce a larger brood size and lower sex ratio. However, this contention cannot be proven with the existing data.

#### DISCUSSION

Upon encountering a host that has already been parasitized, a female wasp faces (at least) three 'decisions', (1) whether to superparasitize or not, (2) what number of eggs to lay if she superparasitizes and (3) what sex ratio to produce in the brood. It is evident from this and previous studies that a complicated set of cues is involved in assessing the host to make these decisions. The cues are primarily within the host puparium since a wasp must drill into a host to detect previous parasitization (WHITING, 1967).

WYLIE (1970, 1973) concluded from a series of experiments that the factors responsible for brood size regulation were different from those responsible for the sex ratio change. That conclusion is consistent with results from this study. A wasp can detect previous parasitization even if the attack occurred only 3 hours previously. However, the wasp responds by increasing the sex ratio, and only slightly by decreasing brood size. Oviposition restraint is not strongly expressed unless the first attack occurred more than 24 hours previously. Wylie believed that host death was primarily responsible for the sex ratio response, whereas "venoms" injected into the host were responsible for oviposition restraint. Although methods are not described, WYLIE (1970) stated that host death (defined by cessation of heart beat) occurs around 24 hours after the wasp stings a host in *Musca domestica*. Host death has not been assessed in *Sarcophaga bullata*, but is likely to take longer due to the greater size of these pupae.

The conclusion that host death is primarily responsible for the sex ratio change is not consistent with my data, since I found a sex ratio change even within three hours after parasitization. In discussing host

death, a distinction must be made between cessation of heartbeat and metabolic death of host cells. Although "venoms" cause cessation of heartbeat, some basic metabolic functions of the cells are maintained (WALKER, 1967). When a wasp stings a host but is removed prior to oviposition, the host can remain in "suspended animation" for over 6 weeks (*pers. obs.*). On the other hand, excessive stinging (e.g. during high wasp densities) will cause cell death and decomposition of the host (WALKER, 1967).

The dramatic changes observed with a 48 hour delay between parasitizations may be due to host death and other changes in the host caused by venom. The amount of venom injected into the host could play an important role in the speed with which cessation of heartbeat occurs. Therefore, the negative relationship between brood size of the 1st and 2nd observed with a 48 hours delay may be related to the amount of venom injected into the host by the first wasp. Given that a wasp stings the host on each successive oviposition bout (EDWARDS, 1954), the amount of venom may be roughly correlated with egg number. A low amount of venom could delay host death (or other changes) and thus lead to less oviposition restraint. Unfortunately, host death was not measured in these studies.

Oviposition restraint of the second wasp can be separated into two components (1) superparasitism restraint and (2) brood size regulation (given superparasitism). Experiment D shows that superparasitism restraint is strongly affected by the presence of feeding larvae within the host, independent of host death. However, the brood size of superparasitizing wasps is not strongly affected. This result further illustrates the complexity of cues involved in assessing a host. Possible cues associated with feeding larvae could be metabolic waste products, such as CO<sub>2</sub>, or pheromones produced by the larvae.

The general pattern of oviposition restraint makes adaptive sense. Offspring of the first and second broods compete for food within the host. Therefore there is a definite trade off between the number of eggs a female lays in a host and the fitness of her offspring. As time between first and second parasitization increases, the second brood becomes increasingly at a disadvantage with respect to the first due to the accelerating growth of the first brood. Thus the superparasite is favoured to produce a smaller brood size, which she does. Evidence suggests that the superparasite adjust her brood size so as to decrease the negative effects upon the size (and hence fecundity) of her offspring (WERREN, *in prep.*). The brood size of a second wasp (and the probability of superparasitism) should be affected both by the conditions of the host and by her own condition. For example, if the wasp has many mature eggs, then her propensity of superparasitize should increase.

WYLIE (1966) found significant wasp mortality for the egg to adult stage in his experiments, which utilized housefly (*Musca domestica*) hosts. In contrast, WALKER (1967), VELTHUIS *et al.* (1965) and WERREN (1980a, b) found low levels of mortality when blowfly (calliphorid and sarcophagid) host were used, so long as an excessive number of wasps were not confined with hosts. This difference is due primarily to the fact that *N. vitripennis* is poorly adapted to parasitization of houseflies (LEGNER, 1967), and normally parasitizes blowflies in nature (WERREN, 1983; SKINNER, 1983). Although mortality does not typically occur on superparasitized blowfly hosts (when the wasp is allowed free movement), a reduction in the size of male and female offspring does occur in the superparasite brood. Offspring size is not reduced in the first brood (WERREN, ms.). Assuming that female progeny suffer (in fitness) relatively more by the reduction in size, then host quality is another selective factor favoring male biased sex ratios in superparasitized hosts. Host quality alone is not adequate to explain the superparasite response, however. Superparasite offspring size is reduced but does not vary with her brood size, which would be necessary to explain the negative relationship between brood size and proportion sons produced by the superparasite. The pattern is predicted by the relative local mate competition model (WERREN, 1980a). The fact that offspring size does not vary with brood size suggests that the wasp adjusts her brood size so as to decrease negative effects among offspring.

In certain other wasp species, mortality is a common result of superparasitism. This is especially true of solitary species, in which only a single offspring can develop within a host (VINSON, 1976; LAWRENCE, 1981). Even in gregarious species, mortality can occur and would presumably be especially pronounced when only a few offspring (e.g. 1-5) can typically develop in a host ("semi-gregarious" species, *sensu* WERREN, 1984) or when multiple superparasitism occurs.

It is tempting to speculate on the coevolution of 1st wasp and 2nd wasp brood size strategies in *N. vitripennis*. The brood size of the first wasp is known to increase with host size in *N. vitripennis* (WYLIE, 1967). For any given host size, the optimal number of eggs laid by a primary parasite will be influenced by the relationship between egg number and offspring fitness (WAAGE & MING, 1984), which is in turn influenced by the probability of superparasitism and the likely brood size of the superparasite. If the primary parasite lays too many eggs onto a host, then the presence of a 2nd brood could cause stunting and perhaps mortality of the 1st wasp's offspring. Thus one might expect the 1st wasp to "hedge her bets" by laying fewer eggs into a host than could be supported by the food available. As anecdotal evidence, when



wasps are allowed to freely parasitize hosts in an area (WERREN, 1980b), there is typically a significant amount of food remaining within the host after development of the progeny. If the host is superparasitized, the food reserve is reduced, indicating that the reserves could have successfully supported more first wasp progeny, probably without stunting. Another explanation for this pattern is that the wasp is simply "spreading risk" by laying eggs in many hosts.

The second wasp should generally restrict egg number so as to prevent stunting of both her own offspring and those of the first wasp. Recall that the first wasp daughters are potential mates for the 2d wasp's sons. The situation is further complicated if a superparasite can assess the brood size of the 1st wasp and adjust her own brood size accordingly. A complicated "game" might then evolve between first and second wasp strategies. For example, it could be selectively advantageous for the first wasp to lay more eggs into a host (or make it appear so), if this would discourage superparasitism. On the other hand, if outbreeding is selectively favoured (GRANT *et al.*, 1974), then superparasitism might actually be solicited, by laying fewer eggs.

The data showing a negative relationship between first and second brood sizes (with a 48 hour delay between parasitizations) would at first suggest that *N. vitripennis* can assess the brood size of the 1st wasp. The effect is, however, somewhat of an artifact of the experimental design. In the experiment, wasps were permitted to parasitize hosts for only 3 hours, and in many cases laid only a few eggs upon the host in that time. When single wasps are permitted to freely parasitize dispersed hosts (WERREN, 1980a, b) they typically lay many more eggs per host than observed in these experiments. Those hosts with abnormally few 1st wasp eggs are responsible for the negative relationship between 1st and 2nd brood sizes (at 48 hours delay). If only those hosts with a first wasp brood size greater than 15 are analyzed (9570 of freely parasitized hosts have more than 15 progeny, WERREN, 1980b) then the negative relationship disappears. Therefore, although wasps can detect large differences in 1st wasp brood sizes, the result may be an artifact of experimental design.

The sex ratio response of the second wasp is equally complex. An early cue causes a generalized sex ratio increase. Around 48 hours after the first parasitization there is a second cue (or increase of the first cue) which causes a dramatic change in behavior. Sex ratio becomes strongly negatively correlated with egg number laid by the superparasite.

One explanation for the data in experiments A, B & C is that only some wasps have detected previous parasitization, and other wasps have not. The probability of detecting previous parasitization in

creases with time since the previous attack. I hypothesize that *N. vitripennis* has two basic sex ratio behaviors. There is the "first wasp pattern" which produces a strongly female biased sex ratio, roughly independent of brood size. Then there is the "superparasite pattern", in which the sex ratio of the wasp is negatively correlated with the number of eggs she lays into the host. If she lays a few eggs, they are usually 100% sons, but sex ratio declines with increasing egg number.

With a 3 hour delay, only some wasps have detected previous parasitization, but since oviposition restraint is only weakly expressed, they produce a sex ratio slightly elevated over the control. Why do only some wasps detect parasitization with a 3 hour delay? It has been noted (WHITING, 1967) that superparasites sometimes locate and use the previous sting hole into the puparium. It is possible that wasps which sting at or near the previous site can detect previous parasitization early on. After 48 hours, previous parasitization is easily detected by most wasps regardless of sting site, but by then oviposition restraint is strongly expressed. Therefore there is a negative correlation between sex ratio and brood size.

When utilizing the "first wasp pattern", it is clear that *N. vitripennis* does not lay sons first when ovipositing into a host. Proof of this occurs both in the lack of correlation between brood size and sex ratio of the primary parasite (WERREN, 1980b) and from the experiments described in this paper. Instead there is a slight increase in the probability of laying sons later in the oviposition sequence. I hypothesize that the same cues responsible for this slight elevation cause the early elevation of sex ratios by second wasps.

In the "superparasite pattern", it is less clear whether sons are laid early in the oviposition sequence. The data suggest that the wasp lays either a few eggs and high sex ratio, or more eggs and a lower sex ratio. Thus the sex ratio to be produced is linked to the number of eggs laid by the wasp.

The flexible form of sex ratio control observed in *Nasonia vitripennis* contrasts to the more fixed sex ratio control observed in *Trichogramma evanescens* (WAAGE & LANE, 1984). *T. evanescens* has a relatively fixed pattern of laying males first and then females while parasitizing a patch of hosts. This fixed pattern results in generally adaptive sex ratio behavior. When a wasp is ovipositing singly she produces a female biased sex ratio because she lay many eggs. When several females are ovipositing into a patch, each female lays fewer eggs and a higher sex ratio as predicted by theory (HAMILTON, 1967). Other parasites are also known to lay sons first during oviposition (GREEN *et al.*, 1982). Other species of *Trichogramma* do not show the male first pattern, but may have other relatively fixed sex ratio strategies (FEIJEN &

SCHULTEN, 1981). *N. vitripennis* obviously does not pursue a fixed pattern. Sex ratio control is strongly influenced by a variety of extrinsic factors. The sex ratio control is adaptive, since it results in the relative local mate competition effects predicted by WERREN (1980a) and SUZUKI & IWASA (1980). However, it is unclear why *N. vitripennis* would not have a more fixed pattern of laying sons in an oviposition sequence. GREEN *et al.* (1982) pointed out that under extreme local mate competition, precise sex ratio control is necessary to insure that a son is present to inseminate daughters. Otherwise the daughters would go uninseminated. It is possible that the precise sex ratio is less necessary for *N. vitripennis*. Since this wasp typically lays large numbers of eggs into a host (20-40), it is statistically very likely to lay at least one son into the host, even if the sex ratio pattern is not "precise". The fact that sons are somewhat more likely to be laid later in the oviposition series increases the probability that at least one son will be in the host. In addition, *N. vitripennis* often encounters outbreeding situations in nature (WERREN, 1980b, 1983; SKINNER, 1983), where it is less important to ensure that at least one son is present in a host. *N. vitripennis* encounters a range of population structures, from strict sibling mating to panmixia in large populations. In addition, there is variance in host species, host quality, and previous parasitization, each of which can have an impact upon the adaptive sex ratio (WERREN, 1984). The flexible form of sex ratio control documented, allows the wasp to adjust sex ratio in response to this range of important environmental factors.

Apparently, wasp species can differ greatly in their underlying system of sex ratio control. This is to be expected if sex ratio control is an actively evolving character in parasitic wasps. We would expect that species with large panmictic populations will either have little sex ratio control or will adjust sex ratio in response to host size, quality, or other host characteristics. (CHARNOV *et al.*, 1981). Those species in predominantly sibling mating situations will express a rigid, precise form of sex ratio control. Subsequent shifts in population structure over evolutionary time would cause modification of the existing system. Thus, for example, the origin of the flexible system observed in *N. vitripennis* could either be a sibling mating or a panmictic system.

#### ACKNOWLEDGEMENTS

Thanks are extended to J. van den Assem, S. W. Skinner and J. K. Waage for helpful comments on the manuscript. N. Glenn and G. Jepessen provided excellent assistance in data collection. The work was funded in part by NSF grant DEB 7682011A01 and NIH grant GM0746402, and was conducted at the University of Utah.

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