This is an electronic version of the article published as
King, B.H. and S.W. Skinner. 1991. Proximal mechanisms of sex ratio and clutch size responses of the parasitoid wasp *Nasonia vitripennis* to parasitized hosts. Animal Behaviour 42:23-32.

Proximal mechanisms of the sex ratio and clutch size responses of the wasp *Nasonia vitripennis* to parasitized hosts

BETHIA H. KING* & SAMUEL W. SKINNER
Department of Biology, Indiana University, Bloomington, IN 47405, U.S.A.
*Present address: Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115, U.S.A.

Running headline: King & Skinner: Wasp sex ratio and clutch size

Abstract. Female *Nasonia vitripennis* lay fewer eggs and increase the proportion of male offspring when ovipositing in previously parasitized hosts compared to unparasitized hosts. This study examines the location and nature of the cues that females use in these clutch size and sex ratio decisions. Neither the sex ratio nor the clutch size response relies on chemical cues on, or a hole drilled in, the outer shell of the host (the puparium). Rather, the cues for both responses appear to be associated with the host pupa. Females manipulate clutch size but not sex ratio in response to host death: the number of eggs laid on dead hosts is significantly less than on either live hosts or previously parasitized hosts. In addition, the cues that females use for sex ratio manipulation, but not for clutch size manipulation, are local: sex ratio cues are not detected from the end of the host that is opposite the site of parasitization; clutch size cues are. The cues that females use may constrain their sex ratio and clutch size manipulation abilities.

One major group of models for sex ratio manipulation in response to environmental conditions is the local mate competition models. These models have been studied best in parasitoid wasps (reviewed in Waage 1986; King in press). Most parasitoid wasps have haplodiploid sex determination, which potentially allows females to control offspring sex ratio by controlling egg fertilization. In addition, many parasitoid wasps seem to have the subdivided population structure assumed by local mate competition models. One prediction of these models is that a mother should often produce a greater sex ratio (proportion of sons) when she oviposits in a previously parasitized host than in an unparasitized host (Suzuki & Iwasa 1980; Werren 1980). The strongest support for this prediction comes from the parasitoid wasp *Nasonia vitripennis* (e.g. Holmes 1972; Werren 1980). Female *N. vitripennis* oviposit a greater proportion of sons and

fewer total offspring in response to parasitized hosts (e.g. Wylie 1965; Holmes 1972). This reduction in clutch size presumably is advantageous because of the reduction in resources a previously parasitized host presents for the development of the second female's offspring (Skinner 1985a).

Despite the attention that local mate competition models have received, especially for *N. vitripennis*, the proximal mechanism by which a female determines that a host has been parasitized previously is not well understood. Understanding the proximal mechanism is important because it may suggest constraints on manipulation of sex ratio and clutch size (see Discussion).

How female *N. vitripennis* detect that a host has been previously parasitized was first addressed by Wylie in the 1960's and 1970's (references in Discussion). However, the question was not fully answered despite efforts of Wylie and later investigators. Furthermore, Wylie used *Musca domestica* as hosts. Some investigators have suggested that *N. vitripennis* is not well-adapted to *M. domestica* because it is not a typical host (Legner 1967; Werren 1983; but see Rutz & Scoles 1989). We reexamine the question of how females recognize that a host is parasitized, using a larger host, *Sarcophaga bullata*. *Sarcophaga* spp. are common hosts of *N. vitripennis* (Roberts 1933; Werren 1983). Our study corroborates some earlier findings, contradicts others, and extends our knowledge of the cues involved in the sex ratio and clutch size responses to previous parasitization.

The fly pupae that *N. vitripennis* parasitize (Whiting 1967) are ellipsoid and consist of a hard outer shell called the puparium with a soft fly pupa inside. A female wasp uses her ovipositor to drill through the puparium and penetrate and envenom the pupa, causing its death (Beard 1964; Ratcliffe & King 1967). The female then withdraws her ovipositor and lays multiple eggs on the surface of the pupa within the puparium. Females potentially may detect cues within the puparium with sensillae on their ovipositors (van Lenteren 1981).

Through a series of experiments, we examine 1) whether the cues that females respond to in adjusting sex ratio and clutch size are associated with the puparium or with the fly pupa within the puparium, 2) whether females use puparial cues such as a pheromone left on the puparium by a previous female or the drill hole left by a previous female, 3) whether the pupal cues that females use are associated with a previous female's eggs or sting site, 4) whether host death is an important cue, and 5) how localized the cues are that females use.

GENERAL METHODS

We conducted experiments with laboratory populations of *N. vitripennis* and one of its natural host species, the flesh fly *S. bullata,* (obtained from Grubco, Inc., Hamilton, Ohio). We used females with purple-brown eyes (wildtype, CB+ strain) as the primary parasitoids and females with scarlet eyes (ScDr strain) as the secondary parasitoids (Skinner 1985b). Thus, we could assign offspring to their mother on the basis of eye colour. Developing wasps were maintained under continuous light at 27^{o}C. Shortly before emergence from the pupal skin, the wasps were transferred to room temperature (23-24^{o}C). Scarlet-eyed females were mated within 1-2 days after emergence with

recently emerged virgin males of the same strain. We then gave the female honey for 24 h followed by honey and a host for 24 h or 48 h, depending on the experiment. After this pretreatment, we presented the scarlet-eyed female with an experimental host for 24 h. Scarlet-eyed females that produced no daughters in the pretreatment, indicating unsuccessful mating, were excluded from analyses.

We incubated parasitized hosts at 27°C and determined the clutch size and sex ratio of scarlet-eyed offspring surviving to the pupal stage, excluding diapaused offspring (approximately 1% in any experiment). Some experiments were repeated to look at numbers of eggs laid. Throughout this paper, sex ratios, clutch sizes (number of offspring reaching the pupal stage) and numbers of eggs refer to those of the experimental females, i.e., the scarlet-eyed wasps.

Mean sex ratios and clutch sizes were compared by t-tests and one-way ANOVAS when data were normally distributed and homoscedastic and by Mann-Whitney U-tests and one-way Kruskall-Wallis tests when the data were not. For two-way ANOVAS, square-root transformation on sex ratio was used when it improved normality.

Methods of specific experiments are described below. Common to some of these experiments is the glue-cap technique (modified from Smith 1969). To produce parasitized hosts, we put wildtype females each in a test tube, which was then inverted over a host buried in sand (after Skinner 1985b). These hosts were buried to just below the head, or in some experiments, to an equal distance below the abdomen end. Hosts are often partially buried in the field (Werren 1983). After 24 h, we aspirated off the wildtype female and removed the end, the cap, from the host puparium to reveal the end of the host pupa. We then glued on a replacement puparial cap from the same end of another host (Elmer's stixall R adhesive). Once dry, capped hosts were buried as before to cover the glue joint, exposing just the new cap. By manipulating parasitized and unparasitized pupae and caps in this way, we could present females with hosts possessing or lacking specific cues. A host was presented to a scarlet-eyed female by inverting a test tube with the female over the host.

The effect of the glue-cap technique per se on sex ratio and clutch size was tested twice. Analysis was by two-way ANOVA with capped or intact and previously parasitized or unparasitized as main effects. Sex ratio was transformed by taking the square root. Both times there was a significant effect of parasitization on sex ratio ($F_{1,68}$=47.14, P<0.001; $F_{1,67}$=13.52, P<0.001), but no significant effect of capping ($F_{1,68}$=0.19, P=0.67; $F_{1,67}$=0.87, P=0.35) and no significant interaction ($F_{1,68}$=0.01, P=0.95; $F_{1,67}$=0.12, P=0.74). For clutch size there was a significant effect of parasitization in both tests of the technique ($F_{1,72}$=23.27, P<0.001; $F_{1,70}$=34.23, P<0.001), a significant effect of capping in the first but not the second test of the technique ($F_{1,72}$=5.40, P=0.02; $F_{1,70}$=0.63, P=0.43), and in both tests, no significant interaction ($F_{1,72}$=0.02, P=0.89; $F_{1,70}$=0.12, P=0.73). The lack of a significant interaction in both tests of the technique indicates that any effect of capping does not affect parasitized and unparasitized hosts differently, so the technique is valid for comparative purposes. However, the significant effect of capping in one of the tests indicates that the absolute clutch size values should be viewed cautiously.

PUPARIUM-OR-PUPA EXPERIMENT

Methods

To determine whether the cues that females respond to are associated with the puparium or the pupa within, each female received one of four treatments of capped hosts: an unparasitized cap or a parasitized cap on either an unparasitized pupa or a parasitized pupa. The experiment was done twice.

Results

In both replicates, two-way ANOVA on square root-transformed sex ratio showed a significant effect of pupa ($F_{1,63}$=14.27, P<0.001; $F_{1,69}$=27.55, P<0.001) but not of cap ($F_{1,63}$=3.29, P=0.08; $F_{1,69}$=0.68, P=0.41) and no significant interaction effect between cap and pupa ($F_{1,63}$=1.82, P=0.18; $F_{1,69}$=2.68, P=0.11). Regardless of the cap type, a significantly greater proportion of males emerged from previously parasitized pupae than from previously unparasitized pupae (Table I).

Like sex ratio, clutch size responses appeared to be based primarily on cues associated with the fly pupa (Table I). In both replicates, two-way ANOVA showed a significant effect of pupa ($F_{1,69}$=10.51, P<0.01; $F_{1,71}$=25.61, P<0.001) but not of cap ($F_{1,69}$=2.19, P=0.14; $F_{1,71}$=2.36, P=0.13), and there was no significant interaction ($F_{1,69}$=2.24, P=0.14; $F_{1,71}$=0.43, P=0.51). Regardless of the cap type, a significantly smaller number of offspring were produced in response to previously parasitized pupae than in response to previously unparasitized pupae (Table I).

THE HOST PUPARIUM

The previous experiment suggested that females respond primarily to cues associated with the pupa, not the puparium. To be more certain that pupal cues alone are sufficient, two additional sets of experiments were done, the rinse experiments and the puparium drill hole experiment. The rinse experiments tested whether a chemical cue on the surface of the host puparium was necessary to elicit the response to parasitized hosts. The puparium drill hole experiment examined whether a female's response to parasitized hosts is aided by detection of the previous female's drill hole.

Rinse Experiments

Methods

Intially, responses to parasitized and unparasitized hosts of four types were examined: hosts rinsed in 70% ethanol, tap water, a soap solution followed by a tap water rinse or unrinsed hosts. The hosts were allowed to dry before use. Each female was presented with a single host. If any of the rinses removes the cue that a host is parasitized, for that rinse there should be no significant difference between the unparasitized and parasitized hosts. We subsequently tested a fourth rinse, hexane followed by tap water.

Results

Regardless of rinse treatment, sex ratio was greater and clutch size smaller from parasitized than from unparasitized hosts (Table II).

Puparium Drill Hole Experiment

Methods

We presented each female with a parasitized pupa that had been capped with one of three cap types: a parasitized cap, an unmanipulated unparasitized cap, or an unparasitized cap with a small pin hole to simulate its having been drilled by an ovipositor. The pin hole was formed by gently rotating a #1 insect pin until the tip just went through the puparium. If the first female's drill hole aids the second female's detection of previous parasitization, the second female's sex ratio and clutch size from the pupae with the pin-holed caps should be the same as from the pupae with parasitized caps and significantly greater than from the pupae with unparasitized caps.

Results

There was no significant effect of cap type on either sex ratio or clutch size (Table III).

THE HOST PUPA

Eggs-or-Sting Experiments

Methods

In the eggs-or-sting experiments we looked at the effect of the eggs and the sting of the first female on the second female's response. In the first experiment, we examined how removal of the first female's eggs affected the second female's response. We presented each experimental female with an unparasitized host pupa, a parasitized host pupa or a parasitized host pupa with the eggs removed. For all hosts we had glued on caps from unparasitized hosts. If the response to parasitized pupae does not require the presence of the first female's eggs, then the response to parasitized pupae with eggs removed should be the same as to parasitized pupae with eggs present and significantly different from the response to unparasitized pupae. Offspring were allowed to complete development in the first replicate of this experiment; in the second replicate, offspring were counted in the egg stage.

A second experiment was then done. It was similar to the first but with the addition of a treatment of unparasitized host with eggs on the pupal surface. These eggs were tranferred from a host parasitized by a wildtype female. Thus a fly pupa had eggs or a sting site or both or neither.

Results

In the first experiment, the sting site was sufficient to elicit a sex ratio response. The proportion of sons emerging from parasitized-pupae-with-eggs-removed was significantly greater than from unparasitized-pupae and not significantly different from parasitized-pupae-without-eggs-removed (Table IV).

In the second experiment, a two-way ANOVA showed a significant effect of eggs ($F_{1,63}$=4.08, P=0.05) and no significant effect of sting ($F_{1,63}$=2.39, P=0.13) on the proportion of sons produced (Table V). There was no significant interaction between the effect of eggs and sting ($F_{1,63}$=0.00, P=0.97). Though nonsignificant, the effect of sting on sex ratio in the second experiment was in the same direction as in the first experiment (where the effect was significant).

The effect of sting site on clutch size was not clear cut. In the first experiment, the number of adult offspring from parasitized-pupae-with-eggs-removed was not significantly less than from unparasitized-pupae nor significantly greater than from parasitized- pupae-without-eggs-removed (Table IV). Likewise, there was no evidence that females oviposit significantly fewer eggs in previously-parasitized-pupae- with-eggs-removed than in unparasitized pupae ($t=0.93$, $df=31$, $P=0.18$). In the second experiment, however, both the eggs and the sting site resulted in significant reductions in clutch size ($F_{1,69}=4.27$, $P=0.04$; $F_{1,69}=16.74$, $P<0.001$; Table V). There was no significant interaction between eggs and sting site ($F_{1,69}=0.02$, $P=0.88$). Though the two eggs-or-sting experiments differ in statistical significance, they both exhibit trends in the same direction. This was true for number of adult offspring, as well as when number of eggs was counted in the first experiment.

Pupal Damage Experiment

Methods

The pupal damage experiment tested whether females respond to injury to a host caused by a previous female's ovipositor insertion. After *N. vitripennis* pierces a fly pupa, a dark melanized area forms around the sting site. Melanization also occurs when a pupa is pierced with an insect pin. We compared the responses to 1) unparasitized pupae that had each been pierced with an insect pin, 2) unparasitized pupae, and 3) parasitized pupae with eggs removed. All three treatments used capped pupae, with caps from unparasitized hosts. The separation of this experiment from experiment two of the eggs-or-sting experiments above is for heuristic reasons. Results of treatments two and three were used in both experiments; treatment one was run concurrently.

Results

There were no apparent responses to pin-pierced pupae. Sex ratio and clutch size responses to pin-pierced pupae ($0.13 + 0.012$, $N=19$; $32.9 + 2.62$, $N=19$) were not significantly different from the responses to unparasitized pupae ($t=0.39$, $df=34$, $P=0.35$; $t=0.03$, $df=35$, $P=0.98$). Sex ratios were significantly less and clutch sizes significantly greater from pin-pierced pupae than from parasitized hosts with eggs removed ($t=1.70$, $df=35$, $P=0.05$; $t=3.02$, $df=36$, $P<0.01$).

Host Death Experiment

Methods

Here we tested whether females distinguish between parasitized and unparasitized hosts based on whether or not the host is alive. Females were each presented with a host that was (1) parasitized, (2) unparasitized, (3) unparasitized and killed by freezing at -80°C for about 2 h or (4) unparasitized and killed by heating in 60°C water for about 30 min. Frozen and heated hosts were left at room temperature for about 20 h prior to presentation to a female. These extreme temperatures kill fly pupae (11 of 11 heated pupae, 12 of 12 frozen pupae, 0 of 11 control pupae). Offspring were allowed to complete development in the first replicate of this experiment. In the second replicate, treatment 1 was excluded, and numbers of eggs were counted. Egg counting

was facilitated by partially burying hosts in sand, exposing a small area for oviposition (after Skinner 1985b).

Results

We found no evidence that *N. vitripennis*' sex ratio response to parasitized hosts is based on host death. The response to heat- and cold-killed hosts did not differ in sex ratio ($t=0.01$, $df=31$, $P=0.99$), number of offspring reaching pupal stage ($t=1.20$, $df=37$, $P=0.24$) or number of eggs ($t=0.37$, $df=47$, $P=0.71$), so the two types of hosts were combined. The sex ratio from these dead hosts was not significantly greater than from unparasitized hosts (respectively, $X=0.17$, $+ 0.022$, $N=33$ and $X=0.18$, $+ 0.013$, $N=17$; $t=0.33$, $df=48$, $P=0.37$) but was significantly less than from parasitized hosts ($X=0.48 + 0.055$, $N=20$; $t=5.91$, $df=51$, $P<0.001$).

In contrast, clutch size was affected by host death. There were significantly fewer offspring (pupal stage) from killed hosts ($X=19.0 + 1.94$, $N=39$) than from either live unparasitized hosts ($X=45.1 + 4.92$, $N=20$; $t=5.89$, $df=57$, $P<0.001$) or live parasitized hosts ($X=36.6 + 3.22$, $N=20$; $t=4.94$, $df=57$, $P<0.001$). Likewise, significantly fewer eggs were oviposited in killed hosts than in live unparasitized hosts ($X=11.6 + 1.08$, $N=49$ versus $X=28.6 + 2.51$, $N=26$; $t=7.23$, $df=73$, $P<0.001$).

LOCALIZATION OF CUES

Inverted Host Experiments

Methods

In these three experiments we examined localization of cues by determining whether females detect that a host is parasitized when parasitizing the end of the host opposite the first female's parasitization site. In the first of these experiments, we presented each female with a normal-sized S. bullata host partially buried head-down in sand for 24 h. The female was presented with an unparasitized host, the unparasitized end of a parasitized host or the parasitized end of a parasitized host. (Sex ratio and clutch size responses to the parasitized end of a host do not differ in relation to whether the previously parasitized end of the host is the abdomen end or head end ($t=1.10$, $df=53$, $P=0.28$; $t=0.38$, $df=58$, $P=0.71$).) This experiment was done twice: the first time the offspring were allowed to develop to the pupal stage; the second time they were counted in the egg stage.

To determine whether cues could travel to the other end of the host when they had a shorter distance to travel, in the second and third inverted host experiments, the hosts that we used were, respectively, smaller S. bullata, and the even smaller M. domestica.

Results

When females were exposed to the unparasitized ends of previously parasitized hosts, their sex ratios were significantly less than when they were exposed to the parasitized end of parasitized hosts; their sex ratios were not significantly different than when they were exposed to unparasitized hosts (Table VI). These patterns held regardless of whether the hosts were normal-sized S. bullata, small S. bullata, or M. domestica.

In contrast, females produced significantly fewer offspring in parasitized than in unparasitized hosts even when presented with the unparasitized end of the parasitized hosts (Table VII). This was true regardless of whether the host was a normal-sized S. bullata, a small S. bullata, or a M. domestica. The number of adult offspring produced in response to parasitized hosts did not differ significantly according to whether the unparasitized or parasitized end was exposed for normal-sized S. bullata or for M. domestica. For small S. bullata, significantly more adult offspring were produced in response to the unparasitized end. Consistent with these results for number of adult offspring, in normal-sized S. bullata, significantly fewer eggs were oviposited in the unparasitized end of parasitized hosts than in unparasitized hosts ($X=23.5 + 2.46$, $N=21$; $X=34.9 + 3.05$, $N=22$; $t=2.89$, $df=41$, $P<0.01$).

DISCUSSION

Cues Associated with the Host Puparium

A female's response to a parasitized host appears not to be strongly influenced by cues associated with the host puparium (Table I). Females are equally likely to drill parasitized and unparasitized hosts (Wylie 1965), and we found no evidence that a female's sex ratio or clutch size response to parasitized hosts requires detection of either an external chemical cue or the drill hole of the previous female (Tables II, III). If there is a puparial effect on sex ratio, it is certainly not as strong as the pupal effect.

Nasonia vitripennis's apparent reliance on internal cues to detect previous parasitization contrasts with the use of both external and internal cues in most parasitoids of nonmoving hosts (van Lenteren 1981). Perhaps, for species such as *N. vitripennis* in which females often lay eggs even when a host has been previously parasitized, there is no selection to decide what sex ratio and clutch size to produce prior to the insertion of the ovipositor into the host.

Cues Associated with the Host Pupa

Results of our experiments suggest that one internal cue that females may use is a cue associated with the eggs of the previous female (Table V). Our experiments are less clear cut with regards to the effect of the sting site (Table IV, V). However, Wylie (1965, 1966) demonstrated sex ratio and clutch size responses to sting site by comparing the responses to unparasitized hosts versus hosts in which a previous female had been allowed to sting but not lay eggs. The response of a female to sting site may be due to the wasp venom itself or to other chemical or physical changes associated with venom injection, i.e., changes resulting from a physiological host reaction. Lack of responses to pin-pricked pupae in this study suggests that responses to the sting site are not simply responses to pupal changes resulting from puncture by a wasp ovipositor (e.g. melanization).

Wylie (1965) suggested that host death might cue females that a host had been parasitized. Using *M. domestica*, Wylie (1965, 1973) found that females oviposit a greater proportion of sons and a reduced clutch size when parasitizing hosts killed by extreme temperature than when parasitizing live hosts. We too found a reduction in

number of offspring oviposited on hosts killed by extreme temperature relative to on live hosts. However, the reduction was significantly less than occurred in response to parasitized hosts. This result suggests that the clutch size decreases in temperature-killed and parasitized hosts are responses to different cues.

In contrast to Wylie's findings, we found no difference in sex ratio response between hosts killed by extreme temperatures and live unparasitized hosts; and the sex ratio response to hosts killed by extreme temperatures was significantly less male-biased than the response to parasitized hosts. The reason for the difference between Wylie's (1973) sex ratio findings and ours is not clear. Our hosts were heat-killed in the same way as Wylie's except that we heated our hosts longer to compensate for the larger size of *S. bullata*. In addition to our experiment using *S. bullata*, we tried to replicate Wylie's (1973) findings by using the same host species (*M. domestica*) and heating procedure as Wylie (1973); however, we obtained no wasp offspring from heat-killed or cold-killed *M. domestica.*

Localization of Cues

Though both clutch size and sex ratio responses appear to be based on internal cues, the cues are not identical. At least one of the cues used in the clutch size response to parasitized hosts must be detectable from the unparasitized end of parasitized hosts. Clutch sizes are significantly smaller from the unparasitized end of parasitized hosts than from unparasitized hosts (Table VII; Wylie 1970).

In contrast, our results indicate that the cues used in the sex ratio response are relatively localized. Females did not produce a greater proportion of males in response to the unparasitized end of parasitized hosts than in response to unparasitized hosts (Table VI). This result was unexpected given that females increase their proportion of sons when ovipositing on the unparasitized end of *M. domestica* hosts previously parasitized by either of two parasitoid wasp species of the same family, *Spalangia cameroni* and *Muscidifurax zaraptor* (Wylie 1973).

The sex ratio and clutch size responses of *N. vitripennis* increase in absolute magnitude as the time since the first parasitization increases (at least up to 24 hours) (Wylie 1965; Werren 1984). This increase is consistent with a spread of venom or change in pupal characteristics over time. Our results suggest that the sex ratio cue travels more slowly or not as far as the clutch size cue.

The inability of females always to detect that a host has previously been parasitized may account for some of the large amount of variation which has been observed in sex ratio responses to parasitized hosts (Orzack 1986). In addition, if females sometimes fail to recognize hosts as parasitized, observed sex ratios are expected to be less than those predicted by current local mate competition models. The importance of the localized nature of sex ratio cues under natural conditions depends on what proportion of time females drill away from a previous female's parasitization site. Females sometimes insert their ovipositors into the drill holes of previous females; what proportion of time has not yet been quantified.

The difference in cues used for the sex ratio and clutch size responses indicates that the sex ratio response is not due simply to a sequence effect. In some species, males tend to be oviposited early in an oviposition sequence (Green et al. 1982; Waage

& Lane 1984). By reducing clutch size in previously parasitized hosts, such species automatically oviposit a greater proportion of sons.

Cues that females use may change through time. In our experiments we looked at the response to hosts that had been parasitized one day earlier. It would be interesting to look at hosts that had been parasitized even earlier. For example, Werren (1984) suggests that the presence of the first female's larvae feeding in a host reduces the probability of superparasitism and thus decreases average clutch size.

ACKNOWLEDGMENTS

We thank M. Zellar, J. Dutton, W. Gonzalez and R. Madej for their help with counting and colony maintainence. B. K. thanks R. King for helpful discussions on this work and R. King, V. Naples, S. Scheiner, and C. von Ende for comments on the manuscript. This work was supported by an Indiana University Postdoctoral Fellowship to B. K. and by NSF grant BNS 8820069 to S. S. and B. K.

REFERENCES

Beard, R. L. 1964. Pathogenic stinging of housefly pupae by *Nasonia vitripennis* (Walker). J. Insect Pathol., 6,1-7.

Green, R. F., Gordh, G. & Hawkins, B. A. 1982. Precise sex ratios in highly inbred parasitic wasps. Am. Nat., 120,653-665.

Holmes, H. B. 1972. Genetic evidence for fewer progeny and a higher percent males when *Nasonia vitripennis* oviposits in previously parasitized hosts. Entomophaga, 17,79-88.

King, B. H. In press. Sex ratio manipulation by parasitoid wasps. In Evolution and Diversity of Sex Ratio: Insects and Mites (Ed. by D. L. Wrensch & M. Ebbert). New York: Chapman and Hall.

Legner, E. F. 1967. Behavior changes the reproduction of Spalangia cameroni, S. endius, Muscidifurax raptor, and *Nasonia vitripennis* (Hymenoptera: Pteromalidae) at increasing fly host densities. Ann. Entomol. Soc. Am., 60,819-826.

Lenteren, J. C. van. 1981. Host discrimination by parasitoids. In: Semiochemicals: Their Role in Pest Control (Ed. by D. A. Nordlund, Jones, R. L. & Lewis, W. J.), pp. 153-179. New York: John Wiley.

Orzack, S. H. 1986. Sex-ratio control in a parasitic wasp, *Nasonia vitripennis*. II. Experimental analysis of an optimal sex-ratio model. Evolution, 40,341-356.

Ratcliffe, N. A. & King, P. E. 1967. The "venom" system of *Nasonia vitripennis* (Walker) (Hym.: Pteromalidae). Proc. Roy. Entomol. Soc., Lond., 42,49-61.

Roberts, R. A. 1933. Activity of blowflies and associated insects at various heights above the ground. Ecology, 14,306-314.

Rutz, D. A. & Scoles, G. A. 1989. Occurrence and seasonal abundance of parasitoids attacking muscoid flies (Diptera: Muscidae) in caged-layer poultry facilities in New York. Environ. Entomol., 18,51-55.

Skinner, S. W. 1985a. Clutch size as an optimal foraging problem for insects. Behav.

Ecol Sociobiol., 17:231-238.
Skinner, S. W. 1985b. Son-killer--a third chromosomal factor affecting the sex ratio in the parasitoid wasp *Nasonia* (Mormoniella) *vitripennis*. Genetics, 109,745-759.
Smith, G. J. 1969. Host selection and oviposition behavior of *Nasonia vitripennis* (Hymenoptera: Pteromalidae) on two host species. Can. Entomol., 101,533-538.
Suzuki, Y. & Iwasa, Y. 1980. A sex ratio theory of gregarious parasitoids. Res. Popul. Ecol., 22,366-382.
Waage, J. K. 1986. Family planning in parasitoids: adaptive patterns of progeny and sex allocation. In: Insect Parasitoids. (Ed. by J. K. Waage & Greathead, D.), pp. 63-95. London: Academic Press.
Waage, J. K. & Lane, J. A. 1984. The reproductive strategy of a parasitic wasp. II. Sex allocation and local mate competition in *Trichogramma evanescens*. J. Anim. Ecol., 53,417-426.
Werren, J. H. 1980. Sex ratio adaptations to local mate competition in a parasitic wasp. Science, 208,1157-1159.
Werren, J. H. 1983. Sex ratio evolution under local mate competition in a parasitic wasp. Evolution, 37,116-124.
Werren, J. H. 1984. Brood size and sex ratio regulation in the parasitic wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). Neth. J. Zool., 34,123-143.
Whiting, A. R. 1967. The biology of the parasitic wasp *Mormoniella vitripennis* (=*Nasonia brevicornis*) (Walker). Q. Rev. Biol., 42,333-406.
Wylie, H. G. 1965. Discrimination between parasitized and unparasitized house fly pupae by females of *Nasonia vitripennis* (Walk.) (Hymenoptera: Pteromalidae). Can. Entomol., 97,279-286.
Wylie, H. G. 1966. Some mechanisms that affect the sex ratio of *Nasonia vitripennis* (Walk.) (Hymenoptera: Pteromalidae) reared from superparasitized housefly pupae. Can. Entomol., 98,645-653.
Wylie, H. G. 1970. Oviposition restraint of *Nasonia vitripennis* (Hymenoptera: Pteromalidae) on hosts parasitized by other hymenopterous species. Can. Entomol., 102,886-894.
Wylie, H. G. 1973. Control of egg fertilization by *Nasonia vitripennis* (Hymenoptera: Pteromalidae) when laying on parasitized house fly pupae. Can. Entomol., 105,709-718.

Table I. Mean + SE (N) sex ratio (proportion of sons) and clutch size (number of adult offspring) produced in response to hosts consisting of a previously parasitized or unparasitized pupa with a cap from a previously parasitized or unparasitized host

	Sex ratio		Clutch size	
Replicate of experiment	Unparasitized cap	Parasitized cap	Unparasitized cap	Parasitized cap
Replicate 1				
Unparasitized pupa	0.14 + 0.031 (18)	0.15 + 0.023 (13)	30.7 + 3.71 (19)	21.5 + 3.81 (16)
Parasitized pupa	0.29 + 0.068 (19)	0.46 + 0.076 (17)	16.1 + 2.37 (19)	16.1 + 2.31 (19)
Replicate 2				
Unparasitized pupa	0.18 + 0.020 (19)	0.12 + 0.015 (19)	33.2 + 2.92 (19)	27.4 + 2.64 (19)
Parasitized pupa	0.32 + 0.051 (17)	0.37 + 0.056 (18)	18.1 + 2.68 (18)	15.8 + 2.28 (19)

Table II. Mean + SE (N) sex ratio and clutch size produced in response to previously

unparasitized and parasitized hosts which had been rinsed or unrinsed

	Sex ratio		Clutch size	
Rinse	Unparasitized host	Parasitized host	Unparasitized host	Parasitized host
Unrinsed[a]	0.16 + 0.014 (19)	0.57 + 0.060 (20)	55.6 + 4.26 (20)	28.5 + 3.14 (20)
Water[b]	0.15 + 0.009 (18)	0.71 + 0.057 (19)	48.4 + 4.75 (20)	23.4 + 3.17 (20)
Soap[c]	0.16 + 0.018 (18)	0.75 + 0.060 (16)	47.9 + 4.33 (19)	16.7 + 3.11 (18)
Ethanol[d]	0.19 + 0.023 (18)	0.65 + 0.062 (18)	58.1 + 3.42 (18)	22.1 + 2.66 (19)
Hexane[e]	0.17 + 0.007 (30)	0.69 + 0.052 (30)	29.6 + 3.04 (37)	14.1 + 2.21 (36)

Comparisons of sex ratio and clutch size of unparasitized and parasitized hosts for each rinse by Mann-Whitney U tests and t-tests:
[a]sex ratio: U=372.0, P<0.001; clutch size: t=5.11, df=38, P<0.001
[b]sex ratio: U=342.0, P<0.001; clutch size: t=4.38, df=38, P<0.001
[c]sex ratio: U=287.0, P<0.001; clutch size: t=5.79, df=35, P<0.001
[d]sex ratio: U=308.5, P<0.001; clutch size: t=8.36, df=35, P<0.001
[e]sex ratio: U=868.0, P<0.001; clutch size: t=4.12, P<0.001

Table III. Mean + SE (N) sex ratio and clutch size produced in response to previously parasitized pupae with puparial caps from previously parasitized hosts, unparasitized hosts or unparasitized hosts with a pin hole

	Sex ratio[a]	Clutch size[b]
Unparasitized cap	0.31 + 0.029 (23)	17.2 + 2.17 (24)
Parasitized cap	0.28 + 0.043 (22)	17.9 + 2.00 (24)
Pin-holed cap	0.33 + 0.049 (23)	21.0 + 2.09 (24)

[a] $F_{2,65}=0.35$, P=0.71
[b] $F_{2,69}=0.91$, P=0.41

Table IV. Mean + SE (N) sex ratio and clutch size produced in response to pupa which had previously been unparasitized, parasitized or parasitized but with eggs removed

	Treatment	Sex ratio	Clutch size
#1	Unparasitized pupa	0.11 + 0.015 (22)	19.7 + 2.41 (23)
#2	Parasitized pupa	0.17 + 0.024 (19)	14.2 + 2.38 (22)
#3	Parasitized pupa with eggs removed	0.20 + 0.037 (21)	16.8 + 2.07 (23)

Comparisons of mean sex ratios and clutch sizes by Mann Whitney U-tests and t-tests:

Treatment #1 vs. #3: sex ratio: U=325.0, P=0.01; clutch size: t=0.89, df=44, P=0.19
Treatment #2 vs. #3: sex ratio: U=207.0, P=0.84 clutch size: t=0.84, df=43, P=0.20

Table V. Mean + SE (N) sex ratio and clutch size produced in response to pupa which had previously been unparasitized, parasitized, parasitized but with eggs removed or unparasitized with eggs added

	Sex ratio	Clutch size
Unparasitized pupa	0.12 + 0.015 (17)	32.8 + 3.63 (18)
Parasitized pupa	0.21 + 0.049 (15)	14.1 + 2.80 (18)
Parasitized pupa with eggs removed	0.16 + 0.015 (18)	20.8 + 3.04 (19)
Unparasitized pupa with eggs added	0.17 + 0.018 (17)	27.0 + 2.59 (18)

Table VI. Mean + SE (N) sex ratio produced in response to the unparasitized end of parasitized hosts compared to responses to unparasitized hosts and the parasitized end of parasitized hosts, using three types of hosts

Treatment	Normal S. bullata	Small S. bullata	M. domestica
#1 Unparasitized host	0.21 + 0.029 (26)	0.20 + 0.017 (24)	0.21 + 0.030 (21)
#2 Unparasitized end of parasitized host	0.17 + 0.019 (28)	0.19 + 0.020 (25)	0.27 + 0.059 (15)
#3 Parasitized end of parasitized host	0.49 + 0.057 (27)	0.33 + 0.054 (25)	0.50 + 0.110 (11)
Treatment #1 vs. #2	U=386.0 P=0.70	t=0.10 df=47 P=0.92	t=0.97 df=34 P=0.17
Treatment #2 vs. #3	t=5.29 df=53 P<0.001	U=405.5 P=0.04	t=1.92 df=24 P=0.03

Table VII. Mean + SE (N) number of adult offspring produced in response to the unparasitized end of parasitized hosts compared to responses to unparasitized hosts and the parasitized end of parasitized hosts

Treatment	Normal S. bullata	Small S. bullata	M. domestica
#1 Unparasitized host	19.2 + 1.94 (29)	22.6 + 1.65 (25)	9.3 + 0.98 (23)
#2 Unparasitized end of parasitized host	14.7 + 1.69 (30)	15.0 + 1.61 (25)	4.7 + 0.96 (24)
#3 Parasitized end of parasitized host	11.5 + 1.86 (30)	11.1 + 1.41 (27)	3.5 + 0.97 (25)
Treatment #1 vs. #2	t=1.78 df=57 P=0.04	t=3.32 df=48 P=0.001	t=3.38 df=45 P<0.001
Treatment #2 vs. #3	t=1.27 df=58 P=0.10	t=1.81 df=50 P=0.04	t=0.87 df=47 P=0.19