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Sequence of offspring sex production in the parasitoid wasp *Nasonia vitripennis* in response to unparasitized versus parasitized hosts

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Here I examine the mechanism of maternal sex-ratio manipulation in the parasitoid wasp *Nasonia vitripennis*, a species that has received a lot of attention in testing adaptive sex-ratio models (e.g. Werren 1980, 1983; Orzack et al. 1991). Parasitoid wasp females have the potential to control the sex of their offspring by controlling fertilization: sons develop from unfertilized eggs and daughters from fertilized eggs. In most parasitoid wasps that have been examined, females produce a greater proportion of sons when with other females than when alone (reviewed in King 1987, in press). This sex-ratio pattern is found in both gregarious species (multiple offspring develop per host) and solitary species (one offspring develops per host). In the gregarious parasitoid wasp *N. vitripennis*, females not only produce a greater proportion of sons in the presence of other females than when alone (e.g. Wylie 1965; Werren 1983 and references therein), but also generally produce a greater proportion of sons in response to hosts that have been previously parasitized by other females than in response to unparasitized hosts (e.g. Holmes 1972; Werren 1980). Both patterns of sex-ratio behaviour are consistent with predictions of local mate competition (LMC) theory, a group of adaptive sex-ratio models (Hamilton 1967; Suzuki & Iwasa 1980; Werren 1980). Here I demonstrate that *N. vitripennis*'s different sex-ratio response to parasitized versus unparasitized hosts is associated with a difference in the sequence in which sons and daughters are oviposited. I examine the sequence of offspring sex by determining the relationship between sex ratio and clutch size.

I used laboratory populations of two eye colour strains of *N. vitripennis*, ScDr and CB+ wildtype (Werren 1980), and I used *Sarcophaga bullata* as hosts. Mortality of *N. vitripennis* developing in *S. bullata* is low (Werren 1980) and does not appear to differ between the sexes (Holmes 1970 in 1972). Wasps were maintained at 24-28°C under continuous light.

Experimental females (ScDr) were 1-2 day old and had mated successfully, as indicated by subsequent production of daughters. After mating, each female received

honey for 24 h followed by an unparasitized host for 24 h. After this pre-treatment, each female was given a treatment host, either an unparasitized or a parasitized host. Each parasitized host had been exposed to a mated wildtype female for the previous 24 h and produced at least some wildtype offspring (mean \pm SE = 25.88 ± 1.63 , N = 41). These wildtype females had no prior host experience. I gave experimental females treatment hosts for a few minutes to 2.5 h, in order to produce a range of clutch sizes. I conducted parasitized and unparasitized treatments concurrently to minimize the effects of other potential differences between the treatments (e.g. temperature, humidity, host quality).

The offspring sex ratios and clutch sizes that I report below are those of experimental females. Offspring were counted and classified by sex at the pupal stage, with maternity assigned on the basis of eye colour. I excluded females that produced no offspring, and I excluded one diapaused offspring (out of 1070 total offspring). I compared the slopes of the least squares regressions of sex ratio on clutch size for parasitized versus unparasitized hosts after an arcsine square-root transformation on sex ratio to improve the equality of the variances of the regression error terms (Zar 1984).

Sequence of offspring sex production differed between the unparasitized and parasitized host treatments (Fig.1). In unparasitized hosts, the proportion of sons that females produced increased with clutch size; whereas in parasitized hosts, the proportion of sons decreased (Fig. 1). The difference in slope between the regressions was significant ($t = 2.94$, $df = 81$, $P < 0.005$), even when an outlier was excluded from the unparasitized host treatment ($t = 2.83$, $df = 80$, $P < 0.005$). In unparasitized hosts, the number of daughters increased steadily with increasing clutch size; sons were not produced in clutches of less than four. In contrast, in parasitized hosts, the number of sons increased steadily; daughters were not produced in clutches of less than eight. (Larger sample sizes might reveal some females that produce sons earlier in the sequence in unparasitized hosts or daughters earlier in parasitized hosts.) Offspring sex ratio was more variable in parasitized than in unparasitized hosts (Fig. 1; comparison of residuals $F = 2.86$, $df = 40, 43$, $P < 0.001$).

Based on experiments examining the effects of different parasitization periods and different intervals between parasitizations, Werren (1984) hypothesized that *N. vitripennis* has a different pattern of sex-ratio response for parasitized versus unparasitized hosts. Specifically, he suggested that when a female encounters parasitized hosts, her sex ratio is negatively related to her brood size; whereas when a female encounters unparasitized hosts, her sex ratio is 'roughly independent of brood size.' Consistent with Werren's (1984) suggestion, I found a negative relationship between sex ratio and clutch size for females encountering parasitized hosts. However, contrary to Werren's suggestion, I found a positive relationship between sex ratio and clutch size for females encountering unparasitized hosts. The difference in my and Werren's results may be related to differences in sex-ratio behaviour between the wasp strains we used or to other differences in protocol.

The negative relationship between proportion of sons and clutch size in response to parasitized hosts in *N. vitripennis* may be adaptive, as explained through local mate competition theory (Werren 1980). The adaptive significance of a positive

relationship between proportion of sons and clutch size in response to unparasitized hosts, if there is one, is not clear.

Another factor, besides whether a host has been parasitized previously, that appears to affect *N. vitripennis*'s offspring sex sequence is the delay between the first and second female's parasitization. Werren (1984) found a statistically significant negative relationship between the sex ratio and clutch size of females encountering parasitized hosts when there was a 48 h delay, but not when there was a 3-h, 24-h, or 72-h delay (Werren 1984).

Sequence effects on offspring sex are known for other species of parasitoid wasps besides *N. vitripennis*, and the sequence varies considerably among species (e.g. references in Waage 1986; King 1987). A comparison of sequence response to unparasitized versus parasitized hosts has been made in only one other species of parasitoid wasp besides *N. vitripennis*. Similar to the behaviour of *N. vitripennis*, in the solitary species *Telenomus remus*, a female is more likely to oviposit a son first when given a patch of hosts in which a high proportion of the hosts have been previously parasitized by another female than when given a patch of unparasitized hosts (van Welzen & Waage 1987).

Changing the sequence of son and daughter production is not the only way for females to produce a greater proportion of sons in response to parasitized than unparasitized hosts (or in the presence than in the absence of other females). For example, these sex-ratio differences can result from females ovipositing sons early and ovipositing fewer total eggs in parasitized than in unparasitized hosts (or in the presence than in the absence of other females) (Waage 1982a, b). In the parasitoid wasps *Trichogramma evanescens* and *Telenomus remus*, a greater proportion of sons in the presence of other females results in part from such oviposition of sons early and fewer total offspring (Waage & Lane 1984; van Welzen & Waage 1987).

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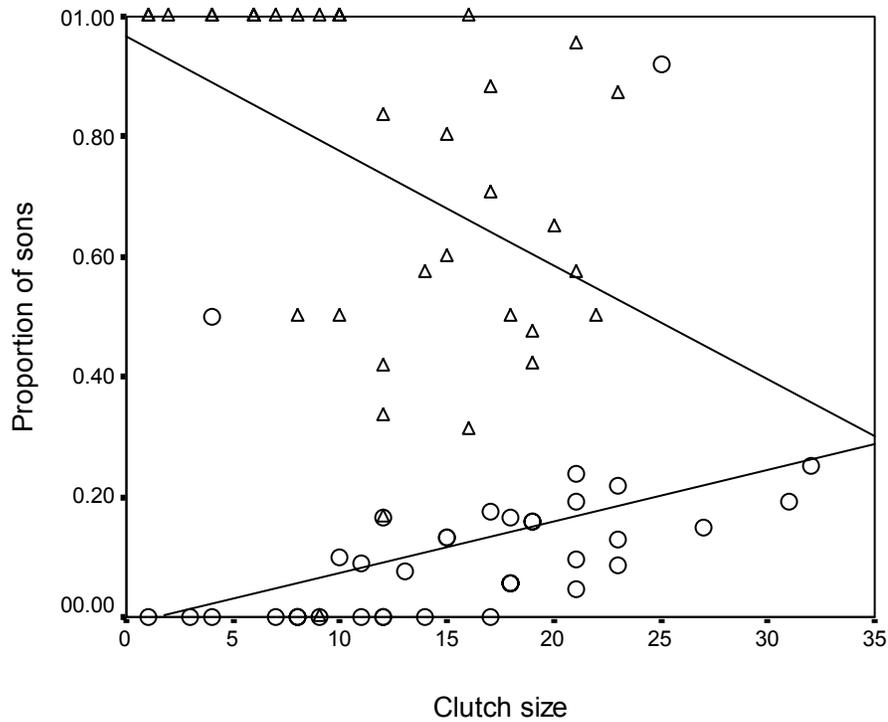


Figure 1. Proportion of sons produced at different clutch sizes in response to unparasitized (○) and parasitized (△) hosts. For unparasitized hosts $y = 0.018x + 0.007$, $R^2 = 0.30$, $F = 17.85$, $df = 1, 42$, $P < 0.0001$ and for parasitized hosts $y = -0.035x + 1.549$, $R^2 = 0.27$, $F = 14.41$, $df = 1, 39$, $P = 0.0003$, where $y = \arcsin$ square root of proportion sons in radians, $x =$ clutch size.