

How smart are *Nasonia* wasps? Limits to the sex allocation response of superparasitizing females

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Nasonia wasps conform to many qualitative and sometimes even quantitative predictions of simple sex allocation models. They are now being used to test more elaborate sex allocation models. In contrast to theoretical predictions, we show that superparasitizing *N. vitripennis* females do not adjust their progeny sex ratio as a function of the sex of eggs laid by the preceding female. The number of offspring of superparasitized females was smaller than that of single females, but we could not determine whether this was due to ovicide or crowding.

Keywords: *Nasonia*, sex allocation, superparasitism, theoretical prediction

The study of sex allocation is considered as one of the success stories of evolutionary biology (Charnov 1982, Werren 1987, Wrensch & Ebbert 1993, Godfray 1994, Hardy 2002, but see Orzack 2002 for a refreshing viewpoint). The success stems from theoretical predictions that have been experimentally verified both qualitatively as well as quantitatively. Many studies have been done with parasitoid wasps because these hymenopterans have haplodiploid sex determination, *i.e.* haploid males developing from unfertilized eggs and diploid females from fertilized eggs. Females store sperm after copulation which apparently enables them to facultatively fertilize an egg or not. *Nasonia vitripennis* (Walker) (Hymenoptera: Chalcidoidea, Pteromalidae), a gregarious pupal parasitoid wasp, has been one of the main species used for testing sex allocation models (*e.g.* Werren 1983, King 2002).

The initial studies of sex allocation have primarily focused on verification of general patterns. Nowadays, we see a tendency towards refinement of theoretical models and experimental testing of the limitations of adaptation. In other words, how good are quantitative predictions really met. For example, Reece *et al.* (2004) and Shuker *et al.* (2004) have recently shown that *N. vitripennis* females are unable to recognize their kin and do not adjust their sex ratio when mating with siblings, although models suggest that such behavior would be advantageous.

In this study we test two additional traits in *N. vitripennis* that may be expected to have evolved in order to optimize sex allocation: the ability of females to

detect the sex of previously laid eggs and their (subsequent) ability to commit ovicide. Hamilton's Local Mate Competition theory predicts that superparasitizing females should adjust their sex ratio according to the number of previous parasitizing females at a particular host. Indeed, females that encounter previously parasitized hosts typically produce a higher proportion of males among their offspring (Werren 1983). This is adaptive, because her sons will favorably compete with sons of the first foundress for mating with the first foundress' daughters. However, not all females in nature are mated and may produce all-male progeny as virgins. If a superparasitizing female is preceded by a virgin female, she would be better off to produce many daughters instead of sons. Several forms of host discrimination have been reported (see Quicke 1997) but these typically involve rejection of females to oviposit in previously parasitized hosts. At a more intricate level, King (2002) presents evidence that suggests that *Spalangia endius* is able to detect the sex of previously laid eggs. The first question we address here is whether *Nasonia* females can determine the sex ratio of a previously laid egg clutch. To answer this question we perform superparasitization experiments in which we manipulate the mating status of the first founding female.

Another adaptive trait that may have evolved under conditions of superparasitism is destruction of eggs (ovicide) of previous foundresses. This will free up resources for the offspring of the superparasitizing female. Ovicide has been reported for the parasitoids *Bracon hebetor* (Strand & Godfray 1989, Antolin *et al.* 1995), *Encarsia* (Arakawa 1987) and *Laelius pedatus* (Mayhew 1997). The second question that we pose is whether *N. vitripennis* females are able to perform ovicide. To answer this question we compare family sizes of superparasitized and non-superparasitized females.

MATERIAL AND METHODS

Nasonia vitripennis parasitizes on various dipteran pupae as *Calliphora* and *Sarcophaga*, in which it can lay 20-40 eggs per host. Females mate once directly after emergence from the pupae and then disperses in search of new hosts. Like all Hymenoptera, it has haplodiploid reproduction; fertilized diploid eggs become female and unfertilized haploid eggs develop into males. It has a short life cycle (± 14 days at 25°C and ± 21 days at 20°C) and can easily be cultured on fly hosts in the laboratory.

Three lines of *Nasonia* were used in our experiments, the laboratory lines AsymC HS (a wildtype line derived from the Leiden LabII line after curing it from *Wolbachia* bacteria) and STDR (a red eye mutant), and the field line HV1 (collected from the Hoge Veluwe in summer 2001 and maintained in diapause since then). The STDR mutant was used to discriminate between the offspring of two females in the same host. Wasps were cultured in plastic tubes (length 6.5 cm, diameter 0.9 cm), closed with cotton wool and provided with 2 or 3 hosts per 2 or 3 days to oviposit on and to feed on. A few days prior to their emergence virgin (unmated) females were collected by dissecting hosts and sexing the pupae.

Table 1. Experimental design of superparasitization. The first female is either producing only males as virgin or a mixed brood when mated. The second female is always mated and can adjust the sex ratio among her progeny in response to the first female. Broods of both females are distinguished based upon eye-colour (STDR is a red-eye mutant). All types of females are also set up as controls (non-superparasitized).

Experimental replicate	First female	Second female
1	Virgin AsymC	Mated STDR
	Mated AsymC	Mated STDR
2	Virgin STDR	Mated AsymC
	Mated STDR	Mated AsymC
3	Virgin STDR	Mated HV1
	Mated STDR	Mated HV1
Controls (first females only)		
Virgin STDR		
Virgin AsymC		
Mated STDR		
Mated AsymC		
Mated HV1		

Collected wasp pupae were kept in groups of 10-15 individuals per tube till emergence. Mated wasps were obtained from hosts that were left untreated. Prior to use in experiments, females were given one host overnight for feeding and oviposition to stimulate egg production. In the experimental design, females were allowed to oviposit for 4 h, after which they were removed from the hosts. The reason for limiting the oviposition window was to prevent crowding and possible differential mortality of the sexes as a result of differences in developmental time and competitive ability.

Experimental design

One experiment was designed to simultaneously test whether *N. vitripennis* females are able to adjust their progeny sex ratio to the sex of eggs laid by previous females, and whether they are able to kill eggs of previous females. Three replicate treatments were performed, each consisting of two females parasitizing the same host in succession. Each pair consisted of one wildtype and one STDR female. The first female was either unmated (producing only unfertilized male eggs) or mated (producing male and female eggs). Unmated and mated females that oviposited singly were used as controls (Table 1). The experiment was repeated on three different days to obtain a sufficient number of replicas (N=45). Cases in which the female (controls) or one of the two females (experiments) had no offspring were excluded from the analysis which resulted in 29-43 replicas per treatment.

Table 2. Average and standard deviations of brood sizes and sex ratios of non-superparasitized control (first) females and superparasitizing (second) females. All broods pooled. Sample sizes between brackets.

Strain	Controls (first females only)		Superparasitizing (second) females	
	Brood size	Sex ratio	Brood size	Sex ratio
Virgin STDR	21.51±8.66 (37)	all-male	n.a.	n.a.
Virgin AsymC	22.36±8.20 (42)	all-male	n.a.	n.a.
Mated STDR	19.05±9.60 (39)	0.12±0.19	13.75±9.80 (79)	0.52±0.34
Mated AsymC	13.00±6.33 (43)	0.12±0.22	11.97±4.95 (64)	0.30±0.32
Mated HV1	16.70±12.19 (40)	0.22±0.24	15.75±8.88 (65)	0.35±0.31

Table 3. Poisson regression analysis of offspring numbers of first females. Both single females and virgin females had more offspring than the average female.

Predictor variable	DF	Coefficient	Chi ²	P
Line	2		28.8	<0.0001
Single	1	0.069	12.7	<0.001
Virgin	1	0.150	62.0	<0.0001

Table 4. Logistic regression analysis of brood sex ratios of second females. The presence of virgin females instead of mated females had no effect, the brood size of the first female had a positive effect and the own brood size had a negative effect on the proportion of sons.

Predictor variable	DF	Coefficient	Chi ²	P
Line	2		139.3	<0.0001
Virgin	1	-0.057	2.1	0.15
Brood size first	1	0.019	103.0	<0.0001
Brood size second	1	-0.052	16.5	<0.0001

Statistics

Statistica 7.0 was used to analyze the data. Offspring numbers were analyzed with Poisson regression, using a log link function and correcting for overdispersion by using the scaled deviance as an estimate of the overdispersion parameter. Sex ratios were analyzed using logistic regression with a logit link function. Significance of variables was tested with standard log-likelihood ratio tests (McCullagh & Nelder 1989).

RESULTS

Brood sizes and sex ratio of non-superparasitized controls

Average brood sizes and sex ratios of non-superparasitized control females are shown in Table 2. Average brood sizes range from 13.0 to 22.4 wasps per host. Broods of virgin females (all-male) are significantly larger than those of mated females (males and females; see Table 3). Brood sizes of mated females differ

between lines, STDR females producing most offspring. All brood sizes are well below the maximum brood sizes that can be obtained from similar sized hosts, indicating that crowding was successfully prevented. Average brood sex ratios (proportion males) of mated females range from 0.12 to 0.22 and differ between lines (Table 4). The HV1 line shows the highest sex ratio.

Effects of superparasitization

Do second females adjust the sex ratio to the presence of first females?

Brood sizes of superparasitizing females ranged from 12.0 to 15.8 wasps per host and were similar to those of first females (Table 2). Sex ratios of second females were significantly more male biased than first females. Moreover, sex ratios decreased with increasing brood size of the second female and increased with the brood size of the first female (Table 3). These results are consistent with LMC theory and confirm previous results (e.g. Werren 1983).

Do second females recognise the sex ratio of first females?

Figure 1 shows the brood sizes and sex ratios of the superparasitizing (second) female as function of whether the first female was virgin (producing only males)

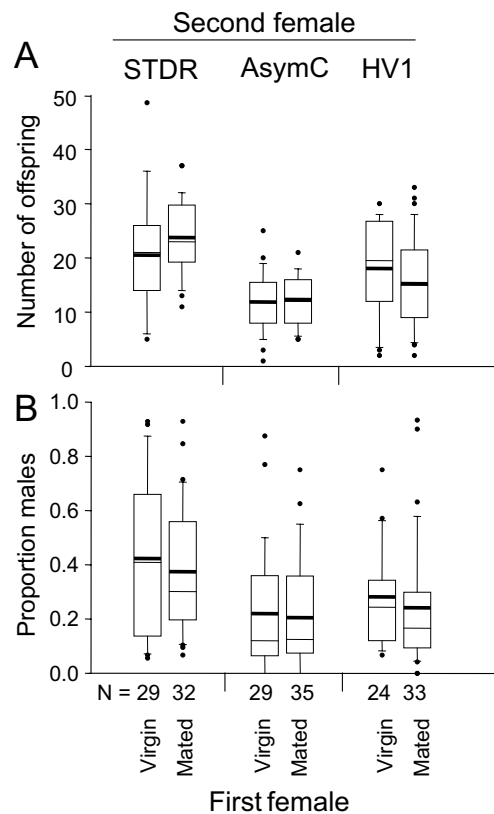


Figure 1. Brood sizes (A) and sex ratios (B) of females superparasitizing clutches of virgin and mated (first) females.

or mated (producing males and females). Neither brood sizes nor sex ratios (Table 4) of second females were different between all-male and mixed first females' broods. Hence, superparasitizing females do not adjust their progeny sex ratios or brood size as a function of the sex ratio of the preceding female.

Do second females reduce brood sizes of first females?

Figure 2 compares brood sizes and sex ratios of the first females after having been superparasitized. Brood sizes of superparasitized clutches are smaller than those from non-superparasitized ones (Table 3). These results indicate either the presence of ovicide in *N. vitripennis* or they could be the result of crowding. Sex ratios of first broods that were later superparasitised are not different from those of single control females (Fig. 3).

DISCUSSION

Nasonia wasps have been used extensively for testing sex allocation theory. After the initial broad confirmation of general predictions, current research is directed towards testing quantitative refinements. Local Mate Competition theory predicts that superparasitizing females produce more males than the initial foundresses. This is adaptive, because the second females gain higher reproductive success through sons than through daughters, because her sons compete with

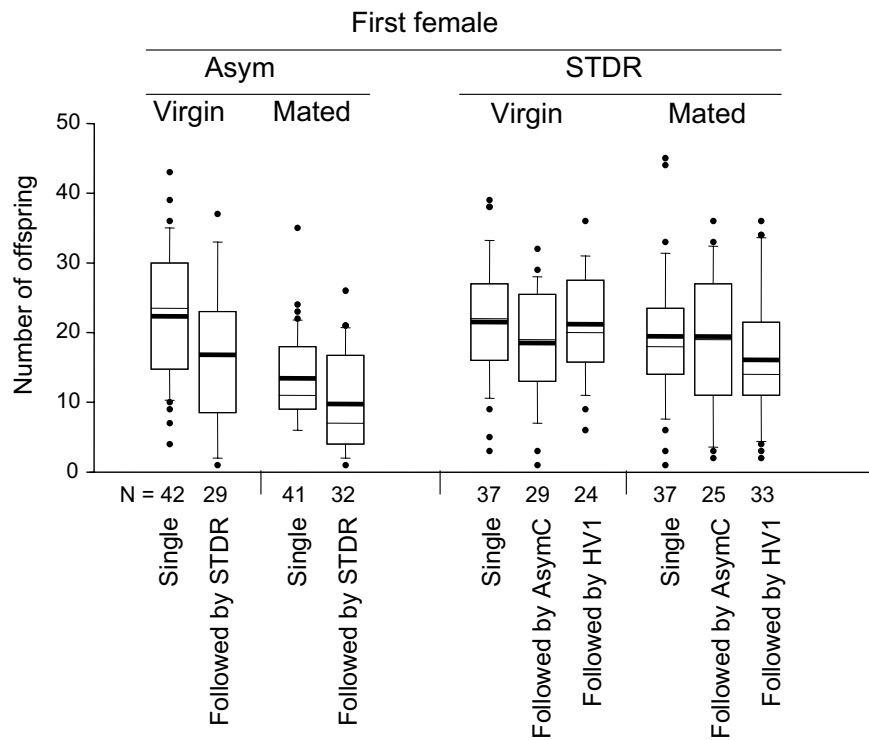


Figure 2. Brood sizes of (first) females superparasitized by a second female.

the first females' sons for access to the first females' daughters. However, when the initial female is unmated she will only produce male offspring. In this case, the superparasitizing females can increase their fitness by producing a high proportion of daughters. Virgin females have been reported to occur at frequencies of up to 12% in natural populations (Beukeboom & Werren 2002). It would therefore pay off for the superparasitizing female to be able to determine the sex ratio of the first clutch and adjust her progeny sex ratio accordingly. Using two laboratory strains and one recently collected field line, we have shown that *N. vitripennis* females produce similar progeny sex ratios when superparasitizing hosts that have previously been parasitized by virgin or mated females. Hence, our data indicate that females are not able to recognize the sex of eggs of previous females.

Another predicted adaptation under LMC theory is the evolution of ovicide; the killing of eggs of previous female. This is advantageous for a superparasitizing female because she can free up more resources for her own offspring by destroying eggs of competing foundresses. Ovicide has been reported for several parasitoids before (Arakawa 1987, Strand & Godfray 1989, Antolin *et al.* 1995, Mayhew 1997). We also found that the presence of a superparasitizing female had a negative effect on the progeny number of the first female. However, we cannot conclude with confidence that *N. vitripennis* wasps commit ovicide since the result might also be due to higher mortality as a result of crowding.

Why do *Nasonia* wasps have these limitations to the adaptive sex allocation response? One explanation may be that the used laboratory lines have lost their

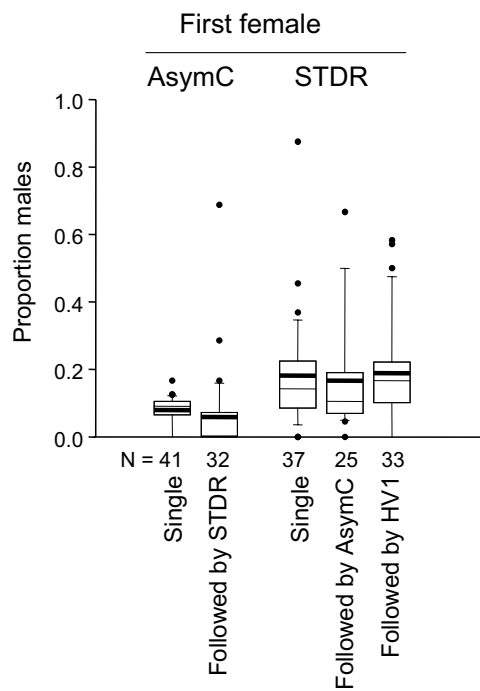


Figure 3. Sex ratios of (first) females superparasitized by a second female.

genetic variation for these abilities. To rule out this possibility we have also used a recently collected field line (HV1) which did not show a different response than the lab lines. A proximate explanation is that they are not able to physiologically determine the sex of eggs, nor are they able to find and/or destroy previously laid eggs. This cannot be the full explanation, because both adaptations have been reported from other parasitoids (King 2002 and abovementioned citations about ovicide). The ultimate explanation must be that natural selection has not been strong enough for these traits to evolve. It could be that conditions of superparasitization are too rare in *Nasonia* for evolution of these adaptations. It is therefore important to obtain more information about the natural population structure of *Nasonia* (cf. Molbo & Parker 1996), in particular to determine superparasitization rates in natural populations. Our results complement previous experiments by Reece *et al.* (2004) and Shuker *et al.* (2004) which showed that *N. vitripennis* females do not adjust their progeny sex ratios according to whether they had mated with sib or non-related males. Altogether, these studies reveal that sex allocation theory is qualitatively and quantitatively supported by experimental evidence in a broad sense, but that specific species may differ in their response at a more refined level.

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