Sib-mating does not lead to facultative sex ratio adjustment in the parasitoid wasp, *Nasonia vitripennis*

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ABSTRACT

Sex ratio theory predicts that in haplodiploid species, females should lay a relatively more female-biased offspring sex ratio when they mate with a sibling compared with when they mate with a non-relative. This is because in haplodiploids, inbreeding leads to females having greater relatedness to daughters relative to sons. This prediction has only been tested in the parasitoid wasp *Nasonia vitripennis*, where no support for this prediction was found. However, a limitation of this previous work is that it was carried out with only two females laying eggs per patch. This is a problem, because in this case the predicted difference in the offspring sex ratio is small and therefore hard to detect. We addressed this problem by utilizing a situation in which larger sex ratio differences are predicted – five females laying eggs per patch. Consistent with the previous results, we also found that the offspring sex ratio laid by a female was not influenced by whether she mated with a sibling or non-relative. Meta-analysis of all the experiments we have undertaken confirms this pattern. This failure to respond to the identity of a mating partner suggests females are unable to discriminate kin and is a relatively rare example of maladaptive sex allocation.

Keywords: kin discrimination, local mate competition, *Nasonia vitripennis*, sex allocation, sex ratio.

INTRODUCTION

Sex ratio theory allows predictions about how natural selection influences life-history evolution to be made and tested quantitatively (Charnov, 1982; West *et al.*, 2000; Hardy, 2002). One of the most productive and successful areas within this field has been Hamilton's theory of local mate competition. Hamilton (1967) showed that when *N* females lay eggs on a patch, and the offspring of these females mate among themselves in their natal patch,

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before the daughters disperse, then the unbeatable or evolutionarily stable offspring sex ratio (proportion of sons) is $r = (N - 1)/2N$. It is now well accepted that local mate competition can explain female-biased sex ratios in numerous animals. For example, local mate competition can explain variation in the sex ratio across populations/species, as well as facultative adjustment of offspring sex ratios by individuals in response to the number of females (N) laying eggs in a patch (Charnov, 1982; Godfray, 1994; Frank, 1998; Hardy, 2002).

In contrast, there is a lack of support for an additional factor that can favour female-biased sex allocation when local mate competition occurs – that is, inbreeding. In species with haplodiploid sex determination, such as wasps and bees, unfertilized eggs develop into males and fertilized eggs develop into females (Cook, 1993). A consequence of this is that inbreeding causes females to be relatively more related to their daughters than their sons (Hamilton, 1972). This can influence the evolutionarily stable offspring sex ratio in two ways. The first possibility is that females adjust their offspring sex ratio in response to the number of females laying eggs per patch, N, and the average level of inbreeding, p, defined as the proportion of individuals that mate with a sibling. In this case, theory predicts that when a given number of females (N) lay eggs on a patch, more female-biased sex ratios are expected in species where there is a higher average level of sib-mating (p) – the evolutionarily stable sex ratio is given by $r = (N - 1)(2 - p)/N(4 - p)$ (Frank, 1985; Herre, 1985). The only support for this prediction comes from comparative studies across fig wasps (Herre, 1985, 1987; Herre *et al.*, 2001). The second possibility is that females adjust their offspring sex ratio in response to whether they have mated with a sibling or non-relative. In this case, theory predicts that split sex ratios (Grafen, 1986) can evolve, with sib-mated females producing a more female-biased sex ratio than females who mate with non-relatives (Greeff, 1996; Reece *et al.*, 2004; Fig. 1). Specifically, if we label the evolutionarily stable sex ratio for a sib-mated female as s_1^*, and for a female who has mated with a non-relative as s_0^*, then if $N < 5$:

$$s_0^* = \frac{(2N - 1)^2(N^2 + 4N - 2)}{(1 - p)N(9N - 4)^2}$$

and   (1)

$$s_1^* = \frac{(2N - 1)^2(5N - N^2 - 2)}{pN(9N - 4)^2}$$

For $N \geq 5$, we get $s_1^* = 0$ and

$$s_0^* = \frac{1}{2(1 - p)} \frac{N - 1}{N}$$ (2)

(Reece *et al.*, 2004). The only test of this prediction has been carried out by Reece *et al.* (2004) with the parasitoid wasp *Nasonia vitripennis*. This wasp is an excellent organism with which to test this prediction because: (a) its biology fits the life history assumed by local mate competition theory; (b) there is considerable work showing that local mate competition influences the offspring sex ratio that females produce; and (c) sib-mating is relatively common, suggesting that there is selection for a facultative response to sib-mating (Werren, 1980, 1983; King and Skinner, 1991; Orzack *et al.*, 1991; Molbo and Parker, 1996; Flanagan *et al.*, 1998). In contrast to the prediction, however, Reece *et al.* (2004) found that
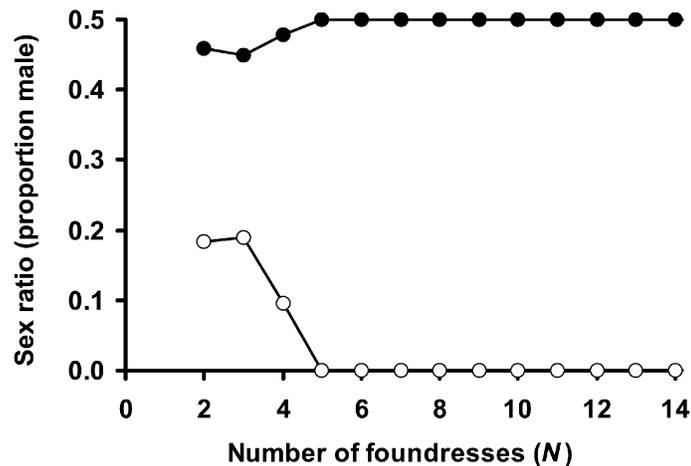
Fig. 1. Predicted optimal sex ratios (proportion male) for females mating with a sibling male (○) or with an unrelated male (●) with respect to foundress number (N). The probability of sib-mating (p) is assumed to be $1/N$ (see Reece *et al.*, 2004).

females did not adjust their sex ratio depending upon whether they mate with a sibling or non-relative, and obtained a negative result. This suggests that females cannot discriminate kin when it comes to mating partners, leading to maladaptive sex allocation under these circumstances. More generally, kin discrimination in non-social Hymenoptera has received remarkably little attention (Fellowes, 1998). However, one possible explanation for this negative result is that Reece and colleagues' experimental design utilized only two females per patch ($N = 2$), and that this situation does not select for sex ratios sufficiently different to be resolved experimentally. With $N = 2$, and assuming $p = 1/N$, theory predicts a sex ratio difference of 0.28 between individuals who mate with a sibling or non-relative (equation 1; Reece *et al.*, 2004).

Here, we address this problem by testing for split sex ratios due to sib-mating in a situation where larger sex ratio differences are expected. Specifically, we examine the off-spring sex ratios produced by individual *N. vitripennis* wasps when five females are allowed to lay eggs per patch ($N = 5$). In this case, assuming $p = 1/N$, theory predicts a sex ratio difference of 0.5 between individuals who mate with a sibling or non-relative (equation 1). This difference is sufficient to provide strong selection for sex ratio adjustment, as well as considerable statistical power in detecting it.

MATERIALS AND METHODS

Study organism

Nasonia vitripennis (Hymenoptera: Chalcidoidea) is a gregarious parasitoid wasp of dipteran pupae, including numerous species of Calliphoridae and Sarcophagidae (Whiting, 1967). Females typically mate once before dispersing to find new oviposition sites. Males have reduced wings and are unable to fly, remaining at the site of adult emergence to compete with each other for matings with emerging females. Females lay clutches of 20–40 eggs and limit oviposition in previously parasitized hosts (superparasitism) if possible.

Two field-collected strains, HV236 and HV307, kindly provided by Professor L. Beuke-boom, were used for the experiment. These were collected from bird boxes at Hoge Veluwe, the Netherlands, in the summer of 2002. In addition, a red-eye mutant laboratory strain, STDR, was used to allow us to identify the broods of individual wild-type females in the multi-foundress groups. All wasps were maintained in mass culture at 25°C under 16 h : 8 h light : dark conditions. Under this regime, males start to emerge after 13–14 days and mate with females who emerge soon after. Both field strains were checked for the absence of sex ratio distorters before the experiments. All wasps were reared on *Calliphora vomitoria* hosts.

Experimental design

For each strain, 300 mated females were randomly selected from mass culture and individually given three fresh hosts in which to oviposit, creating 600 families. After 12 days, the parasitized hosts were isolated and the wasp pupae removed by gently breaking open the host. The pupae were then placed into one of three mating group treatments: (A) 8 sisters and 2 brothers from the same host; (B) 8 sisters and 2 brothers from a different host; (C) 8 sisters and 2 completely unrelated males from the other strain (i.e. HV236 females with HV307 males and vice versa). This sex ratio was chosen as it is typical of sex ratios observed in the field (Molbo and Parker, 1996). In addition, this design means that as well as having any direct genetic cues to use to recognize kin, females had the indirect cue of same versus different host with which to discriminate kin (Reece *et al.*, 2004). Females only provided males and/or females for one replicate mating group. A total of 70 replicate mating groups were set up for each strain.

Following eclosion into adults, females and males in the mating groups were given 48 h in which to mate. One female per replicate was then randomly selected to assay her sex ratio. These females were individually pre-treated for 48 h to allow host-feeding and egg maturation. Pre-treatment consisted of providing each female with a single fresh host for 24 h, and then removing the host and providing honey solution for a further 24 h. Following pre-treatment, each female was placed in a glass tube with five fresh hosts and with four similarly mated and pre-treated STDR females as co-foundresses. After 60 min, a one-way escape tube was fitted to the tube to allow females to disperse after oviposition. After 48 h, all females were removed and the hosts incubated at 25°C and the offspring left to emerge. The number and sex of all experimental wild-type offspring was then recorded, as well as the number of STDR offspring, to control for any influence of relative fecundity on offspring sex ratios (Flanagan *et al.*, 1998). Sex ratio is considered throughout as proportion male. All clutches produced by unmated females (all male broods) were discarded before analysis. In total, clutches from 373 experimental females were sexed and counted, with the sample sizes per treatment ranging from 57 to 67.

Statistical analysis

The sex ratio data were analysed with generalized linear models with binomial errors and a logit link function, using S-Plus 6 (Insightful Corporation). A full model was fitted, including interactions, and then terms deleted in a stepwise fashion to ascertain the minimal adequate model (following Crawley, 2002). Significance was assessed by testing the change in deviance following the removal of a term from the model. After fitting the full model, the data were examined for overdispersion by dividing the residual deviance by the residual

degrees of freedom to give the dispersion parameter; relatively large values of residual deviance suggest overdispersion and risk potential overestimation of the significance level (Crawley, 2002). To account for this, the residual deviance was rescaled by the dispersion parameter and an *F*-test used to determine whether the removal of a term caused a significant increase in deviance. In addition, model fit was checked by examining the distribution of residuals (Crawley, 2002). All other statistics were also carried out with S-Plus 6. Parameter estimates are presented with their standard errors.

RESULTS

There was no significant effect of treatment on the offspring sex ratio ($F_{2,368} = 1.17$, $P = 0.31$; Fig. 2). This pattern held when analysing the two strains separately (HV236: $F_{2,187} = 0.48$, $P = 0.62$; HV307: $F_{2,178} = 1.63$, $P = 0.20$). Consequently, females do not change their sex ratio with respect to the relatedness of their mating partner. A power analysis showed that the minimum significant difference in sex ratio we could resolve as significant between treatment levels was 0.036 or less ($\alpha = 0.05$, power = 0.8), which is much smaller than the differences expected from theory (equations 1 and 2; Fig. 1). Considering other possible explanatory variables, there was a highly significant effect of strain on sex ratio ($F_{1,370} = 94.86$, $P \ll 0.0001$; Fig. 2), a weaker positive effect of focal female clutch size on sex ratio ($F_{1,370} = 7.64$, $P = 0.006$), and no effect of STDR clutch size ($F_{1,367} = 0.47$, $P = 0.49$). The strain effect is comparatively strong, explaining 19.82% of the deviance in sex ratio, with HV236 having a higher mean sex ratio (0.424 ± 0.005) than HV307 (0.304 ± 0.006). The clutch size effect is small, explaining only 1.36% of deviance in sex ratio. Across all treatments, clutch size did not differ significantly between strains (*t*-test: $t_{371} = 0.11$, $P = 0.91$) and clutch size was also not related to STDR co-foundress clutch size (GLM: $F_{1,371} = 0.21$, $P = 0.65$).

All interaction terms were non-significant (all $P > 0.27$), apart from the highest, third-order interaction between all four main effects (treatment × strain × focal female clutch size × STDR clutch size; $F_{2,349} = 3.82$, $P = 0.02$). However, a significance level of $\alpha = 0.005$
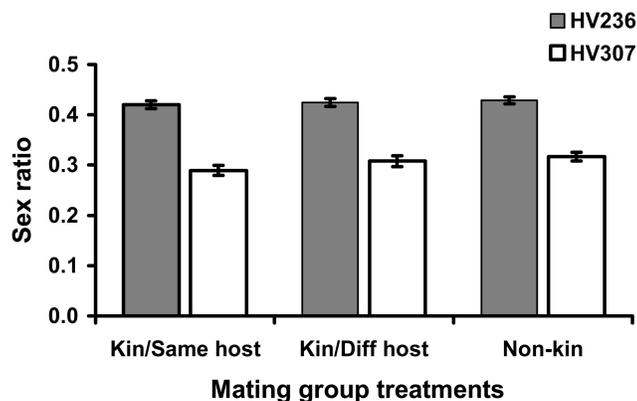
Fig. 2. Sex ratios (proportion male) produced by females with respect to mating group treatment and strain. Standard errors are binomial standard errors. With both strains, females do not lay a significantly different sex ratio dependent upon whether they mate with a sibling or non-relative.

has been suggested as more appropriate for third-order interactions, so as to avoid picking up spurious significant effects with large sample sizes (M.J. Crawley, personal communication). We therefore removed all interaction terms as part of model simplification.

DISCUSSION

We examined the sex ratios produced by individual females of the parasitoid *Nasonia vitripennis* when laying eggs on a patch at the same time as four other females (i.e. $N = 5$). We found no significant effect on the offspring sex ratio of whether a female had mated with a sibling or a non-relative (Fig. 2). This was despite the fact that theory predicts that the sex ratio produced under these circumstances should differ by approximately 0.5 (Fig. 1; Reece *et al.*, 2004). This supports our previous work, which also showed no difference in the sex ratio produced by females who had mated with a sibling or non-relative when two females were laying eggs on a patch (i.e. $N = 2$; Reece *et al.*, 2004). Together, these results provide an extremely strong negative result, suggesting that *N. vitripennis* females do not adjust their offspring sex ratio in response to whether they have mated with a sibling or non-relative. Again, our results reveal differences between strains in sex ratio, indicative of genetic variation in the sex ratio (e.g. Orzack *et al.*, 1991).

We have now carried out 10 distinct tests, in terms of separate strains and experiments, of the hypothesis that sib-mated females should produce a more female-biased sex ratio than females who mated with a non-relative – eight in the study of Reece *et al.* (2004) and two in the present study (Table 1). Consequently, we can also test for any consistent pattern that

Table 1. Summary of statistical tests of how sex ratio varies with relatedness of mating partner, split by experiment and strain

Strain	Test statistic	Effect size (r)	Sample size
HV236[1]	$F_{2,187} = 0.48$, OH test gave $P = 0.20$	+0.06	191
HV307[1]	$F_{2,178} = 1.63$, OH test gave $P = 0.04$	+0.13	182
R6[2a]	$F_{1,99} = 0.07$	−0.03	101
LabII[2a]	$F_{1,96} = 1.19$	−0.11	98
B5[2b]	$F_{2,136} = 3.93$, OH test gave $P = 0.49$	+0.11	139
HV287[2b]	$F_{2,124} = 1.82$, OH test gave $P = 0.18$	−0.08	127
HV55[2b]	$F_{2,159} = 0.19$, OH test gave $P = 0.44$	+0.01	163
HV395[2b]	$F_{2,167} = 1.38$, OH test gave $P = 0.20$	+0.06	171
B5[2c]	$F_{1,174} = 1.01$	+0.08	178
HV287[2c]	$F_{1,165} = 0.22$	+0.04	169

Note: OH gives the test statistic from an ordered heterogeneity test (Rice and Gaines, 1994). The table shows the effect size for the separate (strains/experiments) tests of the hypothesis that females should produce a more female-biased sex ratio when they mate with a relative. The effect size is r, the correlation coefficient, which varies between +1 and −1, with the value of r^2 representing the proportion of variance in the offspring sex ratio that can be explained by the relatedness to a female's mate. The value of r is positive if the correlation is in the predicted direction, and negative if in the opposite direction. Effects sizes (r) were calculated from the statistical tests using standard methodology, described in detail elsewhere (Rosenthal, 1991; Rosenberg *et al.*, 2000). An analysis using standard meta-analysis methods (Rosenthal, 1991; Rosenberg *et al.*, 2000) showed that the average effect size was not significantly different from zero (mean $r = 0.04$, 95% confidence interval = −0.01 to 0.08, $P > 0.07$). More detailed examples and discussion of meta-analysis methodology applied to sex ratio data can be found in West and Sheldon (2002) and Sheldon and West (2004).
[1] This study; [2abc] Reece *et al.* (2004) Experiments 1, 2 and 3, respectively.

is too weak to detect in individual experiments. Overall, there was no consistent trend towards producing more female-biased sex ratios when females mated with siblings: (a) seven out of the 10 studies gave (non-significant) results in the predicted direction, which is not statistically significant with a sign test ($P = 0.34$, two-tailed); and (b) a meta-analysis of the data from the 10 tests showed no significant trend in the predicted direction ($P > 0.05$; Table 1). Future studies should extend empirical work to other species, and test the generality of our results from *N. vitripennis*.

To conclude, we believe that there are two likely explanations for our result that females of the wasp *N. vitripennis* do not produce more female-biased sex ratios when mating with a sibling. First, females may not be able to assess relatedness; there may be difficulties with directly recognizing kin (Grafen, 1990). Indeed, within the social insects, sex ratio shifts in response to relatedness appear to occur via assessment of genetic diversity (which would not be a useful cue in the case examined here for *N. vitripennis*) rather then relatedness *per se* (Keller, 1997). However, factors such as host cues can provide useful indirect cues with which to assess relatedness, and they are used within the parasitoid wasp species *Bracon hebetor* to avoid inbreeding (Ode *et al.*, 1995). Second, adjustment of offspring sex ratios in response to relatedness to mates may not be evolutionarily stable due to conflicts of interest between individuals. Specifically, because males only gain reproductive success through daughters, all males would wish to signal that they are relatives of the female that they are mating, so that the female would produce a female-biased sex ratio. Theoretical analysis of an analogous situation within the social insects has suggested that such conflicts can select against kin recognition mechanisms, removing possibilities for nepotism (Keller, 1997; Reeve, 1998). Although it appears feasible that a similar outcome would arise in the situation examined in this paper, due to conflicts between females and their mates it would be extremely useful to extend theory to specifically address this question.

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