

Kin discrimination and sex ratios in a parasitoid wasp

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Abstract

Sex ratio theory provides a clear and simple way to test if nonsocial haplodiploid wasps can discriminate between kin and nonkin. Specifically, if females can discriminate siblings from nonrelatives, then they are expected to produce a higher proportion of daughters if they mate with a sibling. This prediction arises because in haplodiploids, inbreeding (sib-mating) causes a mother to be relatively more related to her daughters than her sons. Here we formally model this prediction for when multiple females lay eggs in a patch, and test it with the parasitoid wasp *Nasonia vitripennis*. Our results show that females do not adjust their sex ratio behaviour dependent upon whether they mate with a sibling or nonrelative, in response to either direct genetic or a range of indirect environmental cues. This suggests that females of *N. vitripennis* cannot discriminate between kin and nonkin. The implications of our results for the understanding of sex ratio and social evolution are discussed.

Introduction

The evolution of biased sex ratios in spatially structured populations has proved to be one of the most productive areas of evolutionary ecology (Charnov, 1982; Godfray, 1994; West *et al.*, 2000). Hamilton (1967) was the first to show that when the offspring of one or a few mothers mate amongst themselves in their natal patch, before their daughters disperse, a female-biased sex ratio is favoured by natural selection. A useful way of conceptualizing this is that the female bias arises because it reduces competition among a female's sons for mates, and because it increases the number of mates for each of the sons (Taylor, 1981; Frank, 1998). Together these processes have been termed local mate competition (LMC; Hamilton, 1967), and can be formalized with the prediction that the unbeatable sex ratio (proportion of males; r) on a patch is $r = (N - 1)/2N$, where N is the number of foundress females that lay eggs on the patch. There is considerable evidence from a variety of

organisms that this prediction can explain sex ratio variation across species/populations, and also that individuals facultatively adjust their offspring sex ratios in response to the number of females laying eggs per patch (e.g. wasps, ants, beetles, spiders, mites, malaria and related protozoan parasites, snakes and flowering plants; Charnov, 1982; Hardy, 2002).

In contrast, there is a lack of evidence for the importance of an additional factor that can explain sex ratio variation – inbreeding. In haplodiploids, the sex of an egg is determined by whether it is fertilized, with males and females developing from unfertilized (haploid) and fertilized (diploid) eggs respectively. A consequence of this is that inbreeding causes mothers to be relatively more related to their daughters than their sons, and so in haplodiploids, a more female-biased sex ratio is favoured than in diploids (Frank, 1985; Herre, 1985). The combined effects of LMC and inbreeding can be formalized with the prediction $r = (N - 1)/(2 - p)/N(4 - p)$, where p is the proportion of individuals that are sib-mated (Frank, 1985; Herre, 1985; Werren, 1987). The only evidence for the separate effects of LMC and inbreeding come from Herre's work on fig wasps, where for a given number of foundresses (N), sex ratios produced by inbred

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species were more female-biased (Herre, 1985, 1987; Herre *et al.*, 2001). However, Greeff (1996) has shown theoretically that individuals can be selected to facultatively adjust their sex ratio in response to whether they mate with a sibling or a nonrelative. Greeff's (1996) model predicts split sex ratios, with sib-mated (inbred) females producing a more female-biased sex ratio than females who do not mate with sibs (outbred). The pattern found by Herre in fig wasps could therefore be explained either by females adjusting their sex ratio in response to the average level of inbreeding, or females facultatively adjusting their sex ratio in response to sib-mating.

As well as explaining sex ratios, an understanding of whether individuals show facultative adjustment of the sex ratio in response to sib-mating is important for two more general reasons. First, it provides a relatively easy way to examine if nonsocial wasps can discriminate between kin and nonkin (Greeff, 1996; West *et al.*, 2000; West & Herre, 2002). Our understanding of kin discrimination in nonsocial species (and hence its possible importance in the evolution of sociality) is extremely poor, especially when compared with work on social species (Fellowes, 1998). This is largely because the specialized behaviours associated with sociality, such as helping, offer relatively easy ways to test for kin discrimination (Bourke & Franks, 1995; Clutton-Brock, 2002; Griffin & West, 2002). Secondly, it can help explain the controversial genetic variation in behaviour that has been observed in the sex ratio behaviour of parasitic wasps (Orzack *et al.*, 1991; Hardy, 1992), a point that we shall return to in the discussion.

Here, we present the first empirical test of whether individuals facultatively adjust their sex ratio as predicted by Greeff (1996) in response to whether they mate with a sibling. Greeff's (1996) prediction was not developed for parasitoid and fig wasps where multiple females lay eggs per patch (see section 2). Consequently, our first aim is to develop theory that predicts how females should adjust their offspring sex ratios in response to sib-mating when N females lay eggs per patch. Our model is more easily tested in a variety of haplodiploid organisms. For facultative sex ratio adjustment in response to sib-mating to evolve, individuals would have to be able to discriminate between siblings and nonrelatives. Such kin discrimination can occur via direct genetic cues, or via indirect environmental cues. For example, Ode *et al.* (1995) have shown that the parasitic wasp *Bracon hebetor* uses an indirect cue to assess relatedness – females avoid inbreeding by preferring to mate with males that developed in a different host, with host odour rather than genetic relatedness providing the cue for kin discrimination. We carried out three experiments on the parasitoid wasp *Nasonia vitripennis* to determine if individuals adjust their sex ratio in response to whether they mate with a sibling. Inbreeding is relatively common in natural populations of *N. vitripennis* (Molbo & Parker, 1996). We examined behaviour in response to both direct

genetic cues as well as three indirect environmental cues: (i) host developed in; (ii) time between emergence and mating and (iii) sex ratio upon emergence.

Sib-mating and sex ratios

Greeff (1996) has modelled sex ratio behaviour for a situation in which a proportion of an individual's offspring sib-mate and the rest mate with nonrelatives. This model shows that females are predicted to adjust their sex ratio depending on whether they mate with a sibling or nonrelative. Here, we develop theory that allows the level of LMC (N) and the probability of sib-mating (p) to vary independently, and therefore, is more suited to organisms with which this theory can be tested, such as parasitoid or fig wasps. The predictions of our model will differ quantitatively from Greeff's (1996) model because the evolutionary stable sex ratio (ESS) (Maynard Smith, 1982) for a given female will depend not only upon her own mating status (mated with sibling or nonrelative) but also upon the mating status of other females on the patch.

Following the basic life cycle of Hamilton's (1967) original formulation of LMC, we assume that: (i) mated females form groups of variable size (N) in discrete patches where they lay their eggs; (ii) sons and daughters mate at random in their natal patch, after which the newly mated females disperse; (iii) the mating structure, distribution of N , leads to an average probability of sib-mating p . We wish to predict how the sex ratio behaviour of a female should depend upon whether she has mated with a sibling or nonrelative, for given values of N and p . We label the ESS sex ratio for a sib-mated female as s_1^* , and for a female who has mated with a nonrelative as s_0^* . In the appendix we derive the following results. If $N < 5$, then

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

and

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

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

$$s_0^* = \frac{1}{2(1 - p)} \frac{N - 1}{N}. \quad (3)$$

In Fig. 1 we show the predictions for an example when $p = 1/N$. In agreement with Greeff (1996) our model predicts split sex ratios, with sib-mated females producing a more female-biased sex ratio. However, in some cases, our model also predicts a domed relationship between the sex ratio and foundress number (N) for sib-mated individuals. Such nonmonotonic predictions are unusual for LMC models. It arises in this case because for large N , sib-mated females are rare and so they can produce a large bias in their own sex ratio with relatively little effect on the patch sex ratio. In contrast, at low N sib-mated females are more common and can have a large effect on

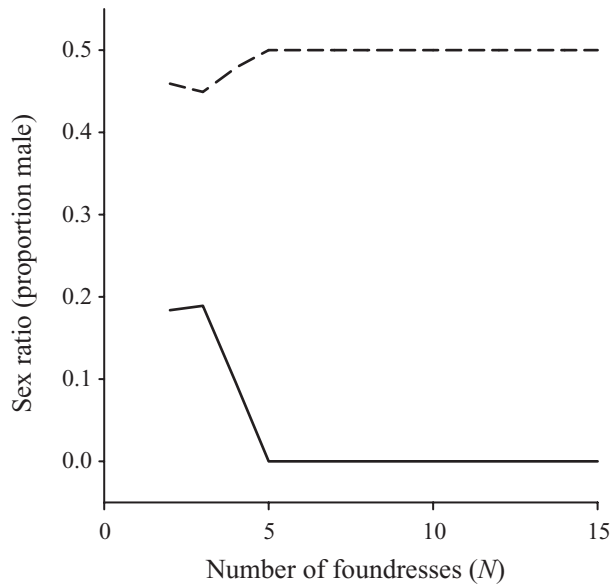


Fig. 1 Inbreeding and facultative sex ratio adjustment. The predicted ESS sex ratios for females who have mated with a sibling (solid line) or a nonrelative (dotted line) is plotted against the number of foundresses (N). The probability of sib-mating (p) is assumed to be $1/N$ for this figure.

the patch sex ratio, decreasing the advantage of producing more daughters.

It is important to note is that selection for split sex ratios will be reduced if there are severe costs of inbreeding (Greeff, 1996). One factor that can lead to appreciable inbreeding depression in haplodiploids is complementary sex determination (CSD) which commonly occurs in social Hymenoptera. In species with CSD individuals that are homozygous at the sex locus develop into males and these diploid males are sterile (Cook, 1993). This leads to inbreeding depression, which is defined as the reduction in fitness of sib-mated offspring relative to outbred offspring. Consequently, the clearest tests of our theory can be carried out in species which do not have CSD, inbreeding depression is relatively low and inbreeding is common. Nevertheless, Greeff (1996) has shown that even when inbreeding depression leads to a decrease in fitness of 0.25 there is a negligible influence on the ES sex ratio. Therefore even in cases where appreciable inbreeding depression occurs, the pattern shown in Fig. 1 can still be expected.

Materials and methods

Study organism

Nasonia vitripennis (Hymenoptera: Chalcidoidea) is a gregarious parasitoid wasp that parasitises a range of dipteran pupae including *Calliphora* and *Sarcophaga* spe-

cies. Female wasps lay clutches of 20–40 eggs in each host and avoid ovipositing in previously parasitised hosts (superparasitism). Females mate once and then disperse to find oviposition sites. Sex allocation in *Nasonia* is well understood, with females responding facultatively to LMC cues (Werren, 1980, 1983, 1984; Orzack & Parker, 1990; King & Skinner, 1991; Orzack *et al.*, 1991; King, 1993; Orzack & Gladstone, 1994; Molbo & Parker, 1996; Flanagan *et al.*, 1998). We cultured wasp lines in 16 h light/8 h dark cycles at 25 °C, in which male offspring emerge after 14 days and mate with females as they emerge the following day. In our three experiments we used six recently isolated field lines; (1) R6 from Rochester, USA, 2000, (2) B5 from Elspeet, Netherlands, 2001, (3) HV287, (4) HV395, (5) HV55 from Hoge Veluwe, Netherlands, 2001 and (6) LabII, an inbred line from Leiden, founded c. 1970. In addition, we used a red-eyed mutant strain (STDR) to allow us to examine the behaviour of individuals when ovipositing in groups. We screened each field line for the absence of sex ratio distorters prior to experiments. Experiment 1 was carried out in March 2000 and experiments 2 and 3 in March 2002. We used relatively large host species for our experiments (*Calliphora vicina* and *C. vomitoria*), to minimize any effect of differential mortality (Werren, 1983).

Experiment 1: sib-mating, host cues and mating delay

In this experiment we simultaneously manipulated whether a female was mating with a sibling or nonrelative and two indirect cues that may indicate sib-mating: (i) host developed in – individuals from the same host are more likely to be siblings than individuals from different hosts, so mating with an individual from the same host may indicate sib-mating (Ode *et al.*, 1995); (ii) delay between emergence and mating – males wait for females to emerge on the host they developed in, so females mating immediately upon emergence are more likely to be sib-mating than females mating after a delay. This experiment consisted of two treatments, each replicated with two different wasp lines, R6 and LabII. In A, the sib-mating treatment, females were mated with brothers that had developed in the same host, and were allowed to mate immediately upon emergence. In B, the nonsib-mating treatment, females mated with a male from the other line who had developed in a different host, and mating was delayed until 48 h after emergence.

For each line we set up 300 singly mated females in individual oviposition patches (tubes containing three hosts). Offspring from each female were used for one mating group replicate only, with one female from each replicate providing sex ratio data, to avoid pseudoreplication (Hurlbert, 1984). We prepared the mating group treatments by removing wasps at the late pupal stage from hosts, approximately 2 days prior to emergence. To set up the sib-mating treatment a single host was placed in a tube to allow the offspring to emerge and mate. To

set up the nonsib-mating treatment we placed five sisters in a tube and added five unrelated males (from the other line) 48 h after the sisters emerged.

We allowed wasps to mate for 48 h in their mating group treatments, by which time all females were mated. One female per mating group replicate was randomly chosen and 'pretreated' individually. This process allows females to host feed and mature eggs and had two stages: (i) placing females in individual tubes with a single host for 24 h; (ii) replacing the host with honey solution for a further 24 h. After pretreatment, each female, together with a red eye mutant marker female (also pretreated), was put into a test tube with eight hosts (hereafter termed the patch) that had a one-way escape tube to allow females to disperse after oviposition and prevent superparasitism (Werren, 1980, 1983, 1984; Godfray, 1994). We removed any females remaining in the patch after 48 h and incubated all clutches at 25 °C. We sexed the offspring of experimental females and also recorded the number of marker female offspring post-emergence to control for any influence of relative fecundity on offspring sex ratios (Flanagan *et al.*, 1998).

Experiment 2: sib-mating and host cues

In this experiment we separately manipulated whether a female was mating with a sibling or nonrelative and an environmental cue, the host developed in. First, we set up mated females to produce full sib families as detailed for experiment 1, using lines B5, HV287, HV 395 and HV 55. This experiment consisted of three mating group treatments: (A) eight sisters and two brothers which developed in different hosts, (B) eight sisters and two males from the other line and (C) eight sisters and two brothers who developed in the same host. The sex ratio of 8 : 2 was chosen to resemble that found in the field (Molbo & Parker, 1996). As in experiment 1, each family provided wasps for one mating group replicate in one treatment. We allowed wasps to mate for 48 h from emergence. Subsequent pretreatment, and collection of sex ratio data were carried out as detailed for experiment 1.

Experiment 3: sib-mating and emergence sex ratio

In this experiment we separately manipulated whether a female was mating with a sibling or nonrelative and an environmental cue that may indirectly suggest sib-mating, the sex ratio upon emergence. If a female emerges into a highly female-biased mating group it may indicate that her group was founded by one or a few females, thus sib-mating is likely. Whereas a mating group with an equal sex ratio suggests multiple founders and a higher probability of mating with a nonrelative. This experiment was carried out using lines B5 and HV287, setting up mated females as previously described. We utilized two treatments (mat-

ing with a sib or nonrelative from the other line), each with two levels (female-biased or equal sex ratio), giving four groups:

(A) sib-mate and female bias (eight sisters and two brothers); (B) sib-mate and equal sex ratio (five sisters and five brothers); (C) nonsib-mate and female bias (eight sisters and two unrelated males from the other line); (D) nonsib-mate and equal sex ratio (five sisters and five unrelated males from the other line). We allowed wasps to mate for 48 h from emergence. Subsequent pretreatment, and collection of sex ratio data were carried out as detailed for experiment 1.

Analysis

We discarded clutches produced by unmated females (all male offspring) from the analysis. Sex ratio data usually have non-normally distributed error variance and unequal sample sizes. This can be accounted for by assuming binomial errors and a logit link function in a general linear model analysis of deviance (whilst retaining maximum statistical power (Crawley, 1993)). Using S-Plus 6 (Insightful Corporation, Seattle, WA, USA), a full model was fitted, including interactions, and terms deleted in a stepwise fashion (Crawley, 2002). Significance was assessed by examining the change in deviance following removal of each term from the minimal model. After fitting the full model we compared the residual deviance and residual degrees of freedom. Relatively large values of residual deviance indicate overdispersion and potential overestimation of the significance level. To account for this the residual deviance is rescaled by the Heterogeneity Factor (HF; ratio of residual deviance to degrees of freedom), and consequently, an *F*-test was used to test whether the removal of a term caused a significant increase in deviance.

Results

Experiment 1: sib-mating, host cues and mating delay

There was no significant effect of treatment ($F_{1,194} = 0.78$, n.s., HF = 3.86) or the clutch sizes of both the marker females ($F_{1,195} = 1.33$, n.s.) and experimental females ($F_{1,196} = 1.69$, n.s.) on sex ratio. Line R6 had a significantly higher sex ratio than LabII ($F_{1,197} = 128.23$, $P < 0.0001$; see Fig. 2). In addition, the sex ratio of the 'family' each female came from did not influence offspring sex ratio ($F_{1,193} = 0.40$, n.s.), consequently this data was not collected in subsequent experiments.

Experiment 2: sib-mating and host cues

Treatment did not have a significant effect on sex ratio ($F_{2,593} = 1.37$, n.s., HF = 4.52; Fig. 3). There was a significant effect of line on sex ratio ($F_{3,595} = 8.76$,

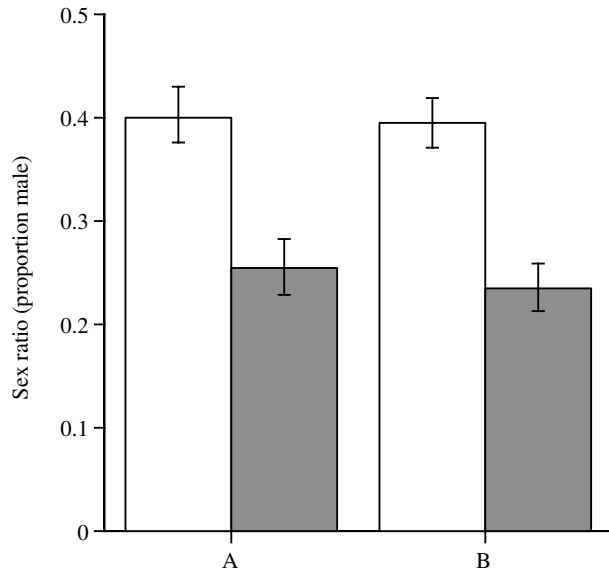


Fig. 2 The mean sex ratio for lines R6 (unshaded) and LabII (shaded) for each treatment in experiment 1. Treatments are represented by A: sib-mating and B: nonsib-mating. Bars are 95% confidence intervals.

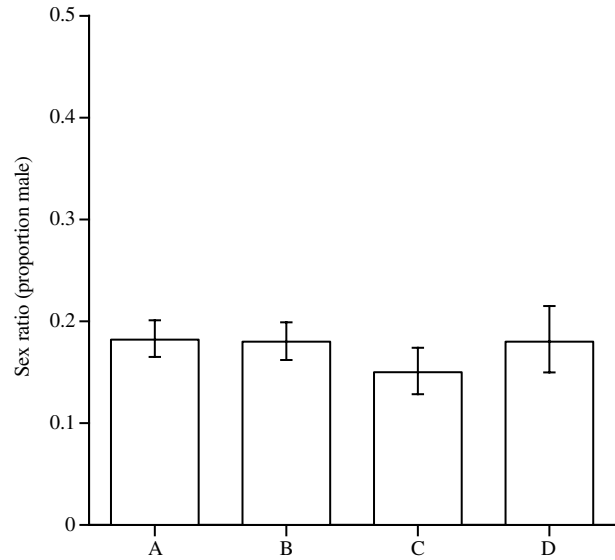


Fig. 4 The mean sex ratio for each treatment in experiment 3, as there was no significant difference between the sex ratios produced by each line their data has been amalgamated. A: sib-mate and female bias, B: sib-mate and equal sex ratio, C: nonsib-mate and female bias and D: nonsib-mate and equality. Bars are 95% confidence intervals.

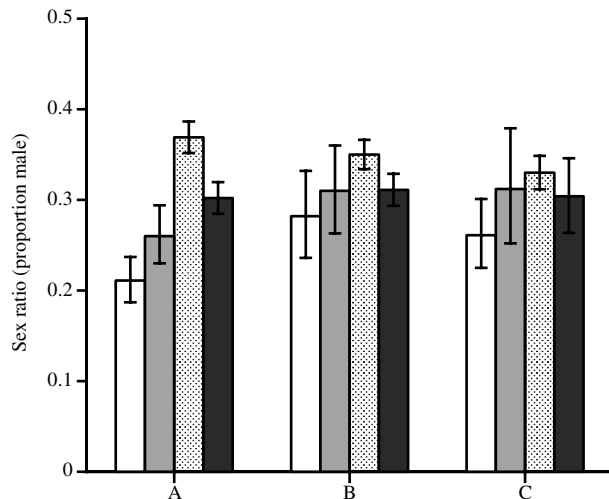


Fig. 3 The mean sex ratio for each treatment in experiment 2, for all lines. For each treatment lines are B5, HV287, HV395 and HV55 from left to right. Treatments consist of A: siblings developing in different hosts, B: nonsiblings and C: siblings developing in the same host and removed prior to emergence. Bars are 95% confidence intervals.

$P < 0.001$; means: HV395 = 0.35; HV55 = 0.31; HV287 = 0.30 and B5 = 0.25; Fig. 3), a weak positive effect of marker female clutch size ($F_{1,595} = 6.68$, $P = 0.01$) and no significant effect of experimental female clutch size ($F_{1,592} = 0.18$, n.s.).

Experiment 3: sib-mating and emergence sex ratio

Marker female clutch size had a significant positive effect on offspring sex ratio ($F_{1,345} = 8.06$, $P < 0.01$, HF = 2.83). Line and experimental female clutch size did not have significant effects on sex ratio ($F_{1,341} = 0.04$, n.s. and $F_{1,342} = 0.21$, n.s. respectively). Neither mating group sex ratio or mate relatedness had a significant effect on sex ratio ($F_{1,343} = 0.26$, n.s. and $F_{1,344} = 1.06$, n.s.; see Fig. 4).

Power analyses

For each experiment we performed a power analysis to explore how big a difference in sex ratio we could detect between treatments (using S-Plus 6; Insightful Corporation). From the theory outlined in section 2, the predicted difference in sex ratio between sib-mated and outbreeding females, in a two foundress patch, is 0.276. For all three experiments, the power to detect a significant difference in sex ratio between treatments of this magnitude was >0.99 , with alpha set at 0.05. The minimum significant difference we could detect between treatments in each experiment was 0.025 or less (with $\alpha = 0.05$, and power = 0.8).

Discussion

We have shown that when multiple females lay eggs on a patch, females are expected to adjust their offspring sex ratio depending upon whether they mate with a sibling

or nonrelative, producing a more female-biased sex ratio when mating with a sibling (Fig. 1; section 2; extending Greeff, 1996). However, in contrast to this prediction, females of the parasitoid *N. vitripennis* did not adjust their sex ratio depending upon: (i) whether they mated with a sibling or nonrelative, or (ii) several environmental cues that may suggest a high or low likelihood of mating with a sibling (host developed in, time between emergence and mating, sex ratio upon emergence). This suggests that females cannot use direct genetic or indirect environmental cues to discriminate kin from nonkin. An alternative explanation is that the underlying theory is incorrect, however the overwhelming success in applying LMC models to *N. vitripennis* makes this explanation unlikely.

Our results have two implications for our understanding of sex ratio behaviour in haplodiploids. First, in fig wasps, more inbred species are observed to have more female-biased sex ratios (Herre, 1985, 1987). If fig wasps also cannot discriminate between kin, then this pattern must be explained by selection on females to adjust their offspring sex ratios in response to the average level of inbreeding in their population. Secondly, much debate has focused on understanding the variation in offspring sex ratios produced by *N. vitripennis* females when ovipositing under the same conditions (Orzack & Parker, 1990; Orzack *et al.*, 1991; see also Fig. 3 for repeatable between line differences in sex ratio). This variation could arise if some females were sib-mated and produced different sex ratios in response to this cue – however, our experiments suggest that this explanation is unlikely. Nonetheless, inbreeding could still help maintain genetic variation if the amount of inbreeding varies spatially or temporally – i.e. through genotype by environment interactions (see West & Herre, 2002).

Why are female *N. vitripennis* unable to discriminate kin from nonkin? The simplest explanation is that the necessary neural and behavioural machinery has not arisen in *Nasonia*. A more intriguing explanation is suggested by the fact that there may be conflict between females and their mates. A haplodiploid sex determination mechanism means that males can only contribute genes to daughters. Consequently, males that are perceived as siblings (or not perceived as unrelated) by their mates will have more daughters, and hence a higher fitness than males perceived as unrelated. Analogous arguments have been made for conflict over kin recognition within social insect colonies (Keller, 1997; Reeve, 1998).

Clearly more experimental work will be required to test the generality of whether haplodiploid females adjust their offspring sex ratios in response to mating with siblings (Greeff, 1996; section 2). Molecular markers such as microsatellites would enable such studies on natural populations. One interesting study from this point is that of Roeder *et al.* (1996) on the mite *Tetranychus urticae*. They showed that females produced a more female-

biased sex ratio when they were related to the other females laying eggs on the patch, and argued that their data supported theory which predicts this pattern (Frank, 1985, 1986; Taylor & Frank, 1996; Courteau & Lessard, 2000). However, Roeder *et al.*'s (1996) experimental treatments confounded the relatedness between females with whether they mated with a sibling or nonrelative. Consequently, their result could also be explained by the effect of sib-mating, as described in section 2.

We conclude with two general points that arise from our observation that *N. vitripennis* females cannot discriminate kin from nonkin mates. First, the absence of kin discrimination in a species of Hymenoptera is not inconsistent with the observation in social insects that workers adjust the sex ratio of reproductives in response to their relative relatedness to males (brothers) and females (sisters; Chapuisat & Keller, 1999; Sundstrom & Boomsma, 2000). The reason for this is that workers appear to assess genetic variability within a colony and adjust their behaviour accordingly, rather than assessing genetic relatedness directly (Keller, 1997). Secondly, if kin discrimination is not common in the Hymenoptera, then the evolution of kin selected social behaviour in the hymenoptera is more likely to have arisen through limited dispersal making individuals interact with relatives (Hamilton, 1964, 1972). Although limited dispersal can also lead to increased competition between relatives, negating such selection for altruism (West *et al.*, 2001, 2002) the life cycle of many Hymenoptera may avoid this problem by a dispersal phase that separates altruism from competition (Queller, 1992; West *et al.*, 2002) and examining this problem in facultatively social species remains a major task.

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References

- Bourke, A.F.G. & Franks, N.R. 1995. *Social Evolution in Ants*. Princeton University Press, Princeton, New Jersey.
- Chapuisat, M. & Keller, L. 1999. Testing kin selection with sex allocation data in eusocial Hymenoptera. *Heredity* **82**: 473–478.
- Charnov, E.L. 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton.
- Clutton-Brock, T.H. 2002. Breeding together: kin selection and mutualism in cooperative vertebrates. *Science* **296**: 69–72.
- Cook, J. 1993. Sex determination in hymenoptera: a review of models and evidence. *Heredity* **71**: 421–435.
- Courteau, J. & Lessard, S. 2000. Optimal sex ratios in structured populations. *J. Theor. Biol.* **207**: 159–175.
- Crawley, M. 1993. *GLIM for Ecologists*. Blackwell Scientific, Oxford.

- Crawley, M.J. 2002. *Statistical Computing. An Introduction to Data Analysis Using S-Plus*. John Wiley & Sons, Ltd, Chichester, UK.
- Fellowes, M.D.E. 1998. Do non-social insects get the (kin) recognition they deserve. *Ecol. Entomol.* **23**: 223–227.
- Flanagan, K.E., West, S.A. & Godfray, H.C.J. 1998. Local mate competition, variable fecundity, and information use in a parasitoid. *Anim. Behav.* **56**: 191–198.
- Frank, S.A. 1985. Hierarchical selection theory and sex ratios. II. On applying the theory, and a test with fig wasps. *Evolution* **39**: 949–964.
- Frank, S.A. 1986. The genetic value of sons and daughters. *Heredity* **56**: 351–354.
- Frank, S.A. 1998. *Foundations of Social Evolution*. Princeton University Press, Princeton.
- Godfray, H.C.J. 1994. *Parasitoids. Behavioural and Evolutionary Ecology*. Princeton University Press, Princeton.
- Greeff, J.M. 1996. Alternative mating strategies, partial sib-mating and split sex ratios in haplodiploid species. *J. Evol. Biol.* **9**: 855–869.
- Griffin, A.S. & West, S.A. 2002. Kin selection: fact and fiction. *Trends in Ecol. Evol.* **17**: 15–21.
- Hamilton, W.D. 1964. The genetical evolution of social behaviour, I & II. *J. Theor. Biol.* **7**: 1–52.
- Hamilton, W.D. 1967. Extraordinary sex ratios. *Science* **156**: 477–488.
- Hamilton, W.D. 1972. Altruism and related phenomena, mainly in social insects. *Annu. Rev. Ecol. Syst.* **3**: 193–232.
- Hardy, I. 1992. Nonbinomial sex allocation and brood sex-ratio variances in the parasitoid hymenoptera. *Oikos* **65**: 143–158.
- Hardy, I.C.W. 2002. Sex Allocation. In: *Sex Ratios: Concepts and Research Methods* (I.C.W. Hardy, ed.), Cambridge University Press, Cambridge.
- Herre, E.A. 1985. Sex ratio adjustment in fig wasps. *Science* **228**: 896–898.
- Herre, E.A. 1987. Optimality, plasticity and selective regime in fig wasp sex ratios. *Nature* **329**: 627–629.
- Herre, E.A., Machado, C.A. & West, S.A. 2001. In: *Selective Regime and Fig Wasp Sex Ratios: Towards Sorting Rigor from Pseudo-Rigor in Tests of Adaptation. Adaptationism and Optimality* (S. Orzack & E. Sober, ed.), pp. 191–218. Cambridge University Press, Cambridge.
- Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* **54**: 187–211.
- Keller, L. 1997. Indiscriminate altruism: unduly nice parents and siblings. *Trends Ecol. Evol.* **12**: 99–103
- King, B.H. 1993. Sequence of offspring sex production in the parasitoid wasp, *Nasonia vitripennis*, in response to unparasitized versus parasitized hosts. *Anim. Behav.* **45**: 1236–1238.
- King, B.H. & Skinner, S.W. 1991. Proximal mechanisms of the sex ratio and clutch size responses of the wasp *Nasonia vitripennis* to parasitized hosts. *Anim. Behav.* **42**: 23–32.
- Maynard Smith, J. 1982. *Evolution and the Theory of Games*. Cambridge University Press, Cambridge.
- Molbo, D. & Parker, E.D. 1996. Mating structure and sex ratio variation in a natural population of *Nasonia vitripennis*. *Proc. R. Soc. Lond. B* **263**: 1703–1709.
- Ode, P.J., Antolin, M.F. & Strand, M.R. 1995. Brood-mate avoidance in the parasitic wasp *Bracon hebetor* Say. *Anim. Behav.* **49**: 1239–1248.
- Orzack, S.H. & Gladstone, J. 1994. Quantitative genetics of sex ratio traits in the parasitic wasp, *Nasonia vitripennis*. *Genetics* **137**: 211–220.
- Orzack, S.H. & Parker, E.D. 1990. Genetic variation for sex ratio traits within a natural population of a parasitic wasp. *Genetics* **124**: 373–384.
- Orzack, S.H., Parker, E.D. & Gladstone, J. 1991. The comparative biology of genetic variation for conditional sex ratio behaviour in a parasitic wasp, *Nasonia vitripennis*. *Genetics* **127**: 583–599.
- Queller, D.C. 1992. Does population viscosity promote kin selection? *Trends Ecol. Evol.* **7**: 322–324.
- Reeve, H.K. 1998. Game Theory, Reproductive Skew, and Nepotism. In: *Theory and Animal Behaviour* (L.A. Dugatkin & H.K. Reeve, eds), Oxford University Press, New York.
- Roeder, C., Harmsen, R. & Mouldey, S. 1996. The effects of relatedness on progeny sex ratio in spider mites. *J. Evol. Biol.* **9**: 143–151.
- Sundstrom, L. & Boomsma, J.J. 2000. Reproductive alliances and posthumous fitness enhancement in male ants. *Proc. R. Soc. Lond. B* **267**: 1439–1444.
- Taylor, P.D. 1981. Intra-sex and inter-sex sibling interactions as sex determinants. *Nature* **291**: 64–66.
- Taylor, P.D. 1988. Inclusive fitness models with two sexes. *Theor. Popul. Biol.* **34**: 145–168.
- Taylor, P.D. & Frank, S.A. 1996. How to make a kin selection model. *J. Theor. Biol.* **180**: 27–37.
- 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.
- Werren, J.H. 1987. Labile sex ratios in wasps and bees. *Bioscience* **37**: 498–506.
- West, S.A. & Herre, E.A. 2002. Using sex ratios: why bother? In: *Sex Ratios: Concepts and Research Methods* (I.C.W. Hardy, ed.), pp. 399–413. Cambridge University Press, Cambridge.
- West, S.A., Herre, E.A. & Sheldon, B.C. 2000. The benefits of allocating sex. *Science* **290**: 288–290.
- West, S.A., Murray, M.G., Machado, C.A., Griffin, A.S. & Herre, E.A. 2001. Testing Hamilton's rule with competition between relatives. *Nature* **409**: 510–513.
- West, S.A., Pen, I. & Griffin, A.S. 2002. Cooperation and conflict between relatives. *Science* **269**: 71–75.

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Appendix

Life cycle

Mated females form groups of variable size in discrete patches where they lay their eggs. Sons and daughters mate at random in their natal patch whereupon the newly mated females disperse to a random location (island model of dispersal), and the cycle starts again. We want to know how females should adjust the sex ratio of their offspring according to whether they have mated with a sibling or a nonrelative.

Evolutionary equilibrium conditions

We focus on a random patch and a random female in that patch. The subscript i will be used to denote the focal female's mating state: sib-mated ($i = 1$) or not ($i = 0$). The patch contains N females, a proportion p of which is sib-mated. Let s_i denote the proportion of sons in the focal female's clutch and \bar{s}_i the average sex ratio of all state- i females (including the focal female) in the focal patch. The average sex ratio of all females in the patch is then given by $\bar{s} = (1 - p)\bar{s}_0 + p\bar{s}_1$. The focal female's fitness is her contribution to the pool of mated females in the next generation and we denote it by $W_i(s_i, \bar{s})$ to remind us that it depends on her own sex ratio s_i and the average sex ratio \bar{s} in the patch. Total fitness can be decomposed into fitness obtained through daughters (W_{fi}) and through sons (W_{mi}), weighted according to sex-specific reproductive values (v_f for daughters, v_m for sons):

$$W_i(s_i, \bar{s}) = v_f W_{fi}(s_i, \bar{s}) + v_m W_{mi}(s_i, \bar{s}). \quad (\text{A1})$$

The number of mated females obtained through daughters is simply proportional to the number of daughters produced:

$$W_{fi} = 1 - s_i. \quad (\text{A2})$$

The number of females mated by sons equals the number of sons (proportional to s_i) times the average number of mates per son $(1 - \bar{s})/\bar{s}$:

$$W_{mi} = s_i \frac{1 - \bar{s}}{\bar{s}} = s_i \frac{1 - (1 - p)\bar{s}_0 - p\bar{s}_1}{(1 - p)\bar{s}_0 + p\bar{s}_1} \quad (\text{A3})$$

We use the direct fitness approach (Taylor & Frank, 1996) to obtain the selection differentials:

$$\frac{dW_i}{ds_i} = v_f \left[r_{fi} \frac{\partial W_{fi}}{\partial s_i} + \bar{r}_{fi} \frac{\partial W_{fi}}{\partial \bar{s}_i} \right] + v_m \left[r_{mi} \frac{\partial W_{mi}}{\partial s_i} + \bar{r}_{mi} \frac{\partial W_{mi}}{\partial \bar{s}_i} \right], \quad (\text{A4})$$

evaluated at $s_i = \bar{s}_i = s_i^*$. The r_{ji} are the coefficients of relatedness of a state- i mother to her sex- j offspring, and \bar{r}_{ji} are the average coefficients of relatedness of a state- i mother to any sex- j offspring (including her own) born in the focal patch. As we assume that females in the same patch are a random sample of the population at large, we know that $\bar{r}_{ji} = r_{ji}/N$. Working out (A4) then gives

$$\begin{aligned} \left. \frac{dW}{ds_0} \right|_{s_0 = \bar{s}_0 = s_0^*} &= -v_f r_{f0} + v_m r_{m0} \frac{N(1 - \bar{s}^*) - (1 - p)s_0^*/\bar{s}^*}{N\bar{s}^*} \\ \left. \frac{dW}{ds_1} \right|_{s_1 = \bar{s}_1 = s_1^*} &= -v_f r_{f1} + v_m r_{m1} \frac{N(1 - \bar{s}^*) - ps_1^*/\bar{s}^*}{N\bar{s}^*} \end{aligned} \quad (\text{A5})$$

Coefficients of Relatedness

For haplodiploid species, $v_f = 2v_m$ and $r_{mi} = 1$ (e.g. Taylor, 1988). We can arbitrarily set $v_f = 1$, therefore it remains to calculate the r_{fi} . For nonsib-mated females, $r_{f0} = 1/2$ and for sib-mated females we obtain

$$\begin{aligned} r_{f1} &= \frac{\text{Random allele daughter IBD to random allele mother}}{\text{Two random alleles mother are IBD}} \\ &= \frac{3 + 5\bar{F}}{4 + 4\bar{F}} \end{aligned} \quad (\text{A6})$$

where \bar{F} is the average inbreeding coefficient (the probability that 2 alleles at the same locus are identical by descent; IBD). The inbreeding coefficient F_j^i among daughters born in a patch of size N_j is given by

$$F_j^i = \frac{1}{N_j} \left[\frac{1}{2} \left(\frac{1}{2} + \frac{1}{2}\bar{F} \right) + \frac{1}{2}\bar{F} \right] = \frac{1}{4N_j} (1 + 3\bar{F}) \quad (\text{A7})$$

Then the change in the average inbreeding coefficient from one generation to the next is given by

$$\bar{F}' = \frac{\sum q_j N_j F_j^i}{\sum q_j N_j} = \frac{(1 + 3\bar{F})}{4 \sum q_j N_j} \quad (\text{A8})$$

Where q_j is the relative contribution of patches of size j to the next generation pool of mated females ($\sum q_j = 1$). In general, the q_j will depend positively on the number of females produced in patches of size j . However, as larger patches are expected to produce less female-biased sex ratios, the q_j are likely to depend only weakly on the sex ratio. Therefore, in the calculation below we assume that the q_j are in fact independent of the sex ratio. If we write $\bar{N} = \sum q_j N_j$ then the equilibrium ($\bar{F}' = \bar{F}$) average inbreeding coefficient is given by

$$\bar{F} = \frac{1}{4\bar{N} - 3}. \quad (\text{A9})$$

Substitution in (A6) gives

$$r_{f1} = \frac{1}{2} \left(\frac{3\bar{N} - 1}{2\bar{N} - 1} \right). \quad (\text{A10})$$

Solutions

Under random mating, the frequency of sib-mated in patches of size j is $1/N_j$. Thus, if we write $p = 1/\bar{N}$, then p is the harmonic mean frequency of sib-mating. To find the equilibrium sex ratios s_i^* as a function of p and patch size N , we substitute (A10) and the other coefficients in the right-hand sides of equations (A5), set the result equal to zero and solve for the s_i^* .

If we assume that females do not adjust the sex ratio facultatively to their mating-state ($s_0 = s_1 = s$) then we get Herre's (1985) result

$$s^* = \frac{(N - 1)(2 - p)}{N(4 - p)}. \quad (\text{A11})$$

If females do adjust the sex ratio facultatively, we get for $N < (5 - 2p)/(1 - p)$

$$\begin{aligned}s_0^* &= \frac{(2N-1)^2(N^2+4N-2)}{(1-p)N(9N-4)^2} \\ s_1^* &= \frac{(2N-1)^2(5N-N^2-2)}{pN(9N-4)^2}.\end{aligned}\tag{A12}$$

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

$$s_0^* = \frac{1}{2(1-p)} \frac{N-1}{N}.\tag{A13}$$