Behavioral Ecology
doi:10.1093/beheco/arj034
Advance Access publication 25 January 2006

Sex ratios under asymmetrical local mate competition in the parasitoid wasp *Nasonia vitripennis*

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Sex ratio theory has proved remarkably useful in testing the adaptive nature of animal behavior. A particularly productive area in this respect is Hamilton's theory of local mate competition (LMC), which has been extended in numerous directions to include greater biological realism, allowing more detailed tests in specific organisms. We have presented one such extension, termed asymmetrical LMC, which occurs when egg laying by females on a patch is asynchronous, and emerging males do not disperse, resulting in the extent of LMC on a patch varying over time. Our aim here is to test whether the parasitoid wasp *Nasonia vitripennis* responds to variation in the degree of asymmetrical LMC. Specifically, we show that females adjust their offspring sex ratios in response to (1) variation in the amount of asynchrony in emergence between broods on a patch and (2) the number and proportion of previously parasitized hosts on the patch. Our results provide qualitative support for the predictions of theory, suggesting new levels of complexity in the sex ratio behavior of this much-studied organism. However, our results do not always provide quantitative support for theory, suggesting further complexities that must be clarified. *Key words:* adaptation, clutch size, constraints, host choice, sex allocation, superparasitism. *[Behav Ecol 17:345–352 (2006)]*

Sex allocation behavior offers excellent opportunities for determining the extent to which evolutionary theory can explain life-history variation (Charnov, 1982; West et al., 2000). Theoretical models predict how individuals should adjust the proportion of their offspring that are male (the sex ratio) in response to local conditions. These models are particularly testable because they often rely on only a small number of key variables that are relatively easy to measure (Frank, 1998; Herre et al., 2001; Seger and Stubblefield, 1996). This has given workers a broad understanding of sex ratio behavior across species as well as providing some startling clear cases of sex ratio adjustment within species (Charnov, 1982; Godfray, 1994; Hardy, 2002; West and Sheldon, 2002; West et al., 2005). A key task for the future is to develop and test more specific models that can be applied and tested with greater precision in specific situations or species.

In a recent paper, we extended Hamilton's (1967) theory of local mate competition (LMC) to incorporate a factor that may be important in many invertebrates, namely asynchronous emergence of offspring on a patch (Shuker et al., 2005). LMC theory predicts a female-biased sex ratio when mating takes place between the offspring of one or a small number of females, before the female offspring disperse (Hamilton, 1967). This female bias is favored because it reduces competition between brothers for mates and provides more mates for sons (Taylor, 1981). Our extension of LMC theory allowed for the possibility that males will encounter competition from males of broods that emerged sometime earlier and have remained on the patch in order to obtain further mating opportunities. We termed this asymmetrical LMC because the sequential emergence of broods laid by different females leads to males from the "first broods" being able to compete for females of the later brood, but not vice versa.

Our asymmetrical LMC model differs from previous models that assume all emergence is synchronous (e.g., Werren, 1980) or that asynchronous emergence affects all broods equally (e.g., Nunney and Luck, 1988). A novelty of this is that it makes specific predictions about how individual females should vary their offspring sex ratios across different broods (hosts) within a patch. This contrasts with previous theory, which has emphasized variation between females in response to factors such as clutch size or fecundity (Frank, 1985a, 1998; Greeff, 1997; Stubblefield and Seger, 1990; Werren, 1980; Yamaguchi, 1985), immigration status (Taylor and Crespi, 1994), and relatedness to mates (Greeff, 1996; Reece et al., 2004). Nonetheless, it should be emphasized that the predictions of our model also rely on the relative clutch sizes laid by different females, and so it is best viewed as an extension of these models. We have already shown that in the parasitoid wasp *Nasonia vitripennis* the assumptions of our asymmetric LMC model can be met and have confirmed the basic predictions of the model (Shuker et al., 2005).

Here we extend our empirical consideration of asymmetrical LMC and explore to what extent females can respond to variation in asymmetrical LMC. We experimentally manipulate information about asymmetrical LMC likely to be relevant to wasps in the wild, namely, the point in time when a proportion of hosts on a patch were previously parasitized. In our first experiment, we test whether females adjust their offspring sex ratio in an unparasitized host in response to the likely emergence times of a brood that has been previously laid by another female on a different host. The emergence time of the other brood is important because it will be the primary determinant of the amount of asymmetrical LMC (Shuker et al., 2005). As the difference in emergence time between broods

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Received 23 September 2005; revised 3 December 2005; accepted 9 December 2005.

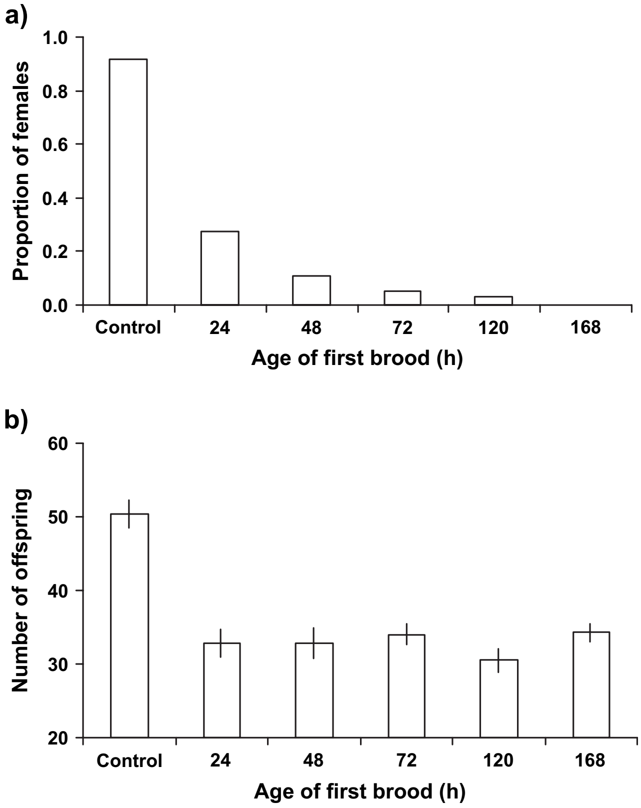

Figure 1
Female oviposition decisions with respect to the age of the brood in the preparasitized host: (a) the proportion of females ovipositing in both hosts and (b) the number of offspring produced by females. In the control treatment, both hosts were unparasitized. In (b) the error bars are standard errors.

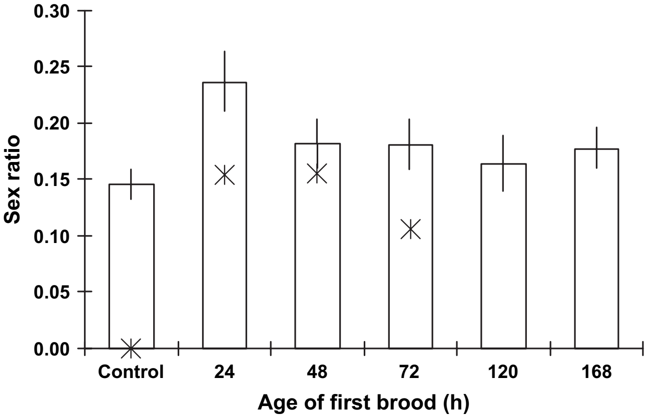

Figure 2
The sex ratio produced by females on patches with respect to the age of the brood in the preparasitized host. The data presented are for females who used unparasitized hosts. In the control treatment, both hosts were unparasitized. The asterisks (*) represent the predicted sex ratio from the asymmetrical LMC model of Shuker et al. (2005) for the degrees of emergence asynchrony for which we can estimate the relative mating abilities of males (up to 72 h asynchrony, see Shuker et al. [2005] for the experiments with the same wasp strains that provided these estimates). The necessary parameters are the clutch sizes of the first and second females (24 h: $c_{11} = 29.97$ and $c_{22} = 32.28$; 48 h: $c_{11} = 29.59$ and $c_{22} = 31.25$; 72 h: $c_{11} = 26.28$ and $c_{22} = 34.03$), the relative competitive abilities of males from one brood competing for females from the other (the relative ability of males from the first female to mate with daughters of the second females: $h_1 = 0.757$, 0.678, and 0.502 for 24, 48, and 72 h asynchrony, respectively; the relative ability of males from the second female to mate with daughters from the first female: $h_2 = 0.192$, 0, and 0 for 24, 48, and 72 h asynchrony, respectively), the sex ratio of the first female's brood ($x_{11} = 0.146$), and the inbreeding coefficient (F, estimated as in Shuker et al. [2005] as 0.31 from Molbo and Parker's [1996] estimate from a wild population). Error bars are 95% confidence intervals.

increases it has two effects, both of which increase the extent of LMC for the second brood, by decreasing the amount of competition with unrelated males: (1) the likelihood that males from the first brood will still be there when the second brood emerges decreases, due to migration and death and (2) the proportion of females in the first brood that can be mated by males from the second brood decreases (Shuker et al., 2005: Figure 1). This leads to the prediction that when there is a previously parasitized host on the patch, the sex ratio produced by a female on an unparasitized host should show a negative relationship with the length of time since the other host was parasitized.

In our second experiment, we test whether females adjust their offspring sex ratio in an unparasitized host in response to the number of parasitized hosts that are also present on the patch. If there are more previously parasitized hosts on a patch, this will increase the number of males that will be competing with the sons of the second female to visit the patch. This reduces the LMC among sons from the second brood, as there will be more unrelated males to compete with, and so should favor a less female-biased sex ratio (Shuker et al., 2005: Figure 2). Consequently, we predict that the sex ratio laid in an unparasitized host should be positively correlated with the number of parasitized hosts on the patch. In addition to our primary consideration of sex ratio, both these experiments also allow us to test several predictions that have been made for how females should vary their host choice and clutch size behaviors (see below).

METHODS

Study organism

The wasp *N. vitripennis* (Hymenoptera: Chalcidoidea) is a gregarious parasitoid of large dipteran pupae (Whiting, 1967). Females lay clutches of eggs on host pupae, with adult males emerging after 13–14 days at 25°C and mating with females that emerge soon after. Females then disperse to find new hosts, while the flightless males remain on the patch. *N. vitripennis* has long been studied in terms of its sex ratio behavior (e.g., Holmes, 1972) and has provided extensive tests of basic LMC theory and extensions thereof (e.g., Flanagan et al., 1998; King et al., 1995; King and Skinner, 1991; Orzack, 1990; Orzack et al., 1991; Shuker and West, 2004; Werren, 1980, 1983, 1984). One important detail is that if superparasitism occurs (a female ovipositing on an already parasitized host), then the offspring from all the broods oviposited on the host emerge relatively synchronously (Werren, 1980), while broods on independent hosts on a patch obviously develop independently. Superparasitism is rare if the period of time between oviposition events is longer than 48 h, due to development and initiation of feeding of the first brood (Werren, 1984, and see below). For the two experiments described below, we used wasps from strain HV6, generated from wasps collected from bird boxes in the field (Hoge Veluwe, Netherlands, summer 2002, by Prof. L.W. Beukeboom). We also used the laboratory red-eye mutant marker strain STDR, which allowed us to generate experimentally parasitized hosts such that we could follow the sex ratio decisions of individual wild-type HV6 females (Werren, 1980).

All wasp cultures were maintained with *Calliphora vomitoria* pupae as hosts, at 25°C, 16:8 h light:dark conditions.

Experiment 1: variation in emergence asynchrony

We allowed females to oviposit on a patch in which there was one unparasitized host and one host which had been previously parasitized by another female (hereafter termed preparasitized). We tested whether a female modifies her oviposition behavior in response to when the previous parasitism had taken place. Our specific predictions are that as the time between the females visiting the patch is increased, the second female should (1) be less likely to oviposit on the parasitized host because it offers a lower quality resource (Charnov and Stephens, 1988); (2) lay a smaller clutch size on the parasitized host (Charnov and Skinner, 1984, 1985; Godfray et al., 1991; Wilson, 1994); and (3) lay a more female-biased sex ratio because the amount of mating between the offspring from the two hosts will decrease (Shuker et al., 2005). This experiment also allowed us to repeat our test of several predictions made in our previous paper (Shuker et al., 2005). These were that the second female should (4) produce a sex ratio negatively correlated with her clutch size relative to the clutch size of the first female; (5) produce a more female-biased sex ratio on the unparasitized host if she lays on both hosts (producing only females on that host in the extreme case); and (6) lay a sex ratio negatively correlated with the first female's clutch size, if she again oviposits on both hosts.

We took 2-day-old mated adult female wasps from HV6 mass cultures and isolated them in 10 × 25–mm vials for pretreatment for 48 h prior to the experiment. Pretreatment consisted of 24 h with a fresh host, followed by 24 h with honey solution. Pretreatment allows host feeding and promotes egg development. After pretreatment, we randomly assigned females to one of six oviposition treatments in which females were given two hosts. These hosts were either two fresh, unparasitized hosts (control—treatment A) or a combination of one unparasitized and one preparasitized host (treatments B–F). We generated preparasitized hosts by giving a single fresh host to a mated and pretreated STDR red-eye mutant female for 24 h. Treatments B–F differed in how long before the experiment hosts were preparasitized, namely (B) 24; (C) 48; (D) 72; (E) 120; and (F) 168 h prior to the experiment. We placed experimental females and hosts in 10 × 75–mm glass vials, and after 1 h we fitted a one-way escape tube to allow females to disperse away from the patch of hosts, to allow females to limit superparasitism (Godfray, 1994; Werren, 1983). After 48 h we removed all females, and each host was put into an individual glass vial and incubated at 25°C. Each host was scored for emerging offspring and the number, sex, and genotype (eye color) of all offspring were recorded. We checked all replicates for the correct preparasitization by STDR females, and we also discarded replicates where females did not appear to have been mated (all-male brood). A total of 276 females were included in the experiment, with sample sizes per treatment ranging from 30 to 59 replicates.

Experiment 2: variation in number of parasitized hosts on a patch

We allowed females to oviposit on a patch on which there was a variable number of unparasitized and preparasitized hosts and tested whether females adjusted their oviposition and sex allocation behavior as predicted. The specific prediction that we tested was that as the number of unparasitized hosts on a patch increases, females should lay increasingly female-biased sex ratios on unparasitized hosts as they will lay more offspring overall and hence be subject to increased LMC (Shuker et al., 2005; Werren, 1980). In addition, we tested predictions (1), (2), (5), and (6) from experiment 1 when appropriate.

We isolated approximately 350 HV6 mated females from mass culture in 10 × 25–mm vials and pretreated them for 48 h with a fresh host followed by honey solution as before. After pretreatment, we randomly assigned females to one of six oviposition treatments: (A) five unparasitized hosts; (B) four unparasitized hosts and one preparasitized host; (C) one unparasitized host and four preparasitized hosts; (D) five preparasitized hosts; (E) two unparasitized hosts; and (F) one unparasitized host and one parasitized host. The last two treatments were included to allow comparison with previous experiments (Shuker et al., 2005), but F also provides an intermediate proportion of hosts on a patch parasitized (i.e., 50%), albeit one confounded by host number; the analysis primarily considers the first four treatments. We again generated preparasitized hosts by giving a single fresh host to a mated and pretreated STDR red-eye mutant female for 24 h immediately prior to the experiment. Experimental females and hosts were placed in 10 × 75–mm glass vials, and after 1 h we fitted a one-way escape tube to allow females to disperse away from the patch of hosts. After 48 h we removed all females, and each host was put into an individual glass vial and incubated at 25°C. We scored each host for emerging offspring and recorded the number, sex, and genotype (eye color) of all offspring. All replicates were checked for the correct number of preparasitized hosts. In total, 338 females provided data on oviposition preferences (sample sizes per treatment 19–96), with 279 females yielding sex ratio data (because not all females chose to oviposit; sample sizes per treatment 11–86).

Statistical analysis

Our general approach for the analysis of sex ratio data is to use generalized linear models with binomial errors and logit link functions and model simplification to identify significant main effects and interactions (Crawley, 2002; Krackow and Tkadlec, 2001; Wilson and Hardy, 2002). Mixed effects models were used where appropriate, with the generalized linear mixed models (GLMMs) performed using partial quasi-likelihood. Throughout we consider sex ratio as proportion male. In addition to exploring the main effects of the experimental manipulations, we also consider other possible sources of sex ratio variation, in particular the influence of relative brood size when focal females are superparasitizing. Theory and empirical data have shown that there is often a negative relationship between relative brood size and sex ratio of the superparasitizing female (Suzuki and Iwasa, 1980; Werren, 1980). Because in both experiments females in some treatments could oviposit in both unparasitized and preparasitized hosts, we consider relative and absolute brood sizes of focal and red-eye females where appropriate. All analyses were performed using S-Plus 6 (Insightful Corporation, Seattle, Washington, USA), with the GLMM command from the MASS library for S-Plus (see http://www.stats.ox.ac.uk/pub/MASS/Windows.shtml; Venables and Ripley, 2002).

RESULTS

Experiment 1: variation in emergence asynchrony

Host choice

As predicted by host choice models, females limited superparasitism when presented with preparasitized and unparasitized hosts, especially as the age of the brood in the preparasitized hosts increased. The proportion of females parasitizing both hosts was negatively correlated with the age of the superparasitized brood ($F_{1,4} = 10.96$, $p = .03$; Figure 1a). The one female who oviposited on a host parasitized 120 h previously did so on a host that produced only one red-eye individual and so presumably only had one or a few red-eye eggs in. No female oviposited in hosts that had been parasitized 168 h previously.

Clutch size

Overall, females in the control treatment (two unparasitized hosts) produced more offspring, corresponding to their greater oviposition in both hosts ($F_{5,252} = 20.30$, $p < .0001$; Figure 1b). However, in terms of the average clutch size in unparasitized hosts, control treatment females produced significantly smaller clutches than in the treatments where only one host was previously unparasitized (Kruskal-Wallis test because variances were heterogeneous: $W_5 = 40.45$, $p < .001$). The clutch size in previously unparasitized hosts did not differ with age of brood across the other treatments (B–F: $F_{1,195} = 0.81$, $p = .37$). Mean clutch sizes in the limited number of hosts that were preparasitized in treatments B–F were small (15.0 eggs per host or less).

Sex ratio

Females varied their sex allocation in response to the age of the preparasitized host on the patch. Specifically, females altered the sex ratio they produced if the preparasitized host had been attacked for the 24 h before the experiment (Figure 2), producing more male brood as a response to the decreased likelihood of LMC among sons. This was true for females that only oviposited on the unparasitized hosts across the treatments ($F_{5,228} = 3.71$, $p = .003$) and also for all females, including those that used the preparasitized host as well ($F_{5,252} = 8.60$, $p < .0001$). In both cases, treatment B females produced less female-biased sex ratios than all the other treatments (which did not differ from each other, $F_{4,228} = 2.05$, $p = .09$; Figure 2), and there was no effect of number of offspring on sex ratio ($F_{1,227} = 0.19$, $p = .66$ and $F_{1,251} = 1.24$, $p = .27$, respectively).

The majority of females that superparasitized were in treatment B (24 h; Figure 1a), with just more than a quarter of those females ($N = 13$) using both hosts. Within this limited number of females, there was a significant difference between the sex ratio produced when using the fresh host (sex ratio = 0.28) and when superparasitizing (sex ratio = 0.54, GLMM with female as random factor: $F_{1,12} = 5.71$, $p = .03$) as expected from theory. There was no association with the number of offspring ($F_{1,12} = 1.13$, $p = .31$) and no effect of red-eye brood size ($F_{1,11} = 0.08$, $p = .78$) or relative brood size ($F_{1,10} = 0.42$, $p = .53$). We therefore found no evidence for the other predictions arising from asymmetrical LMC theory relating to relative clutch sizes, but, due to our focus on testing other factors, the sample sizes are small.

Experiment 2: variation in number of parasitized hosts on a patch

Host choice

Again as predicted by host choice models, females adjusted their oviposition preferences in response to patch composition, preferring to use unparasitized hosts. The number of parasitized hosts on patches with five hosts significantly influenced female oviposition patterns, with females laying eggs in fewer hosts as the number of parasitized hosts per patch increased (comparing the distributions of number of hosts used per treatment: $G_1 = 16.6$, $p < .001$). Figure 3a illustrates the patterns of oviposition in terms of mean number of hosts parasitized. In addition, females on patches with one or four preparasitized hosts (treatments B and C) oviposited nonrandomly, preferentially choosing to oviposit in unparasitized hosts (B: $\chi^2_1 = 18.8$, $p < .0001$; C: $\chi^2_1 = 7.8$, $p < .01$; expected values were calculated using the average number of hosts parasitized in each treatment).

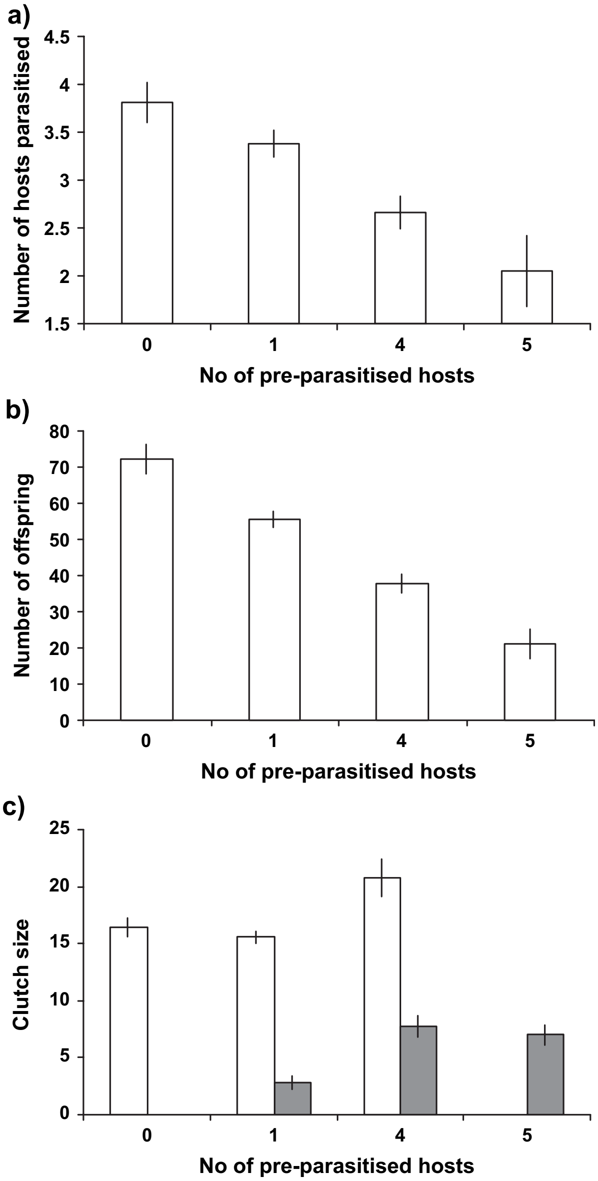

Figure 3
Female oviposition decisions with respect to the number of preparasitized hosts on a five-host patch: (a) mean number of hosts parasitized; (b) mean number of offspring produced on a patch; and (c) mean clutch size in unparasitized (open bars) and preparasitized (shaded bars) hosts. Error bars are standard errors.

Clutch size

Females also adjusted their clutch size behavior in response to patch composition as predicted. At the level of the patch, there was a highly significant difference in total number of offspring produced with respect to treatment, with females producing less offspring as the number of preparasitized hosts increased ($F_{3,194} = 30.60$, $p < .0001$; Figure 3b). In terms of the average clutch size produced on unparasitized hosts, females did not differ in the average number of eggs oviposited per host with respect to whether there were five, four, or one unparasitized hosts on the patch (treatments A–C; data ln(+1) transformed to homogenize variances: $F_{2,181} = 0.03$, $p = .97$). In contrast, there was a significant difference in the clutch sizes laid by

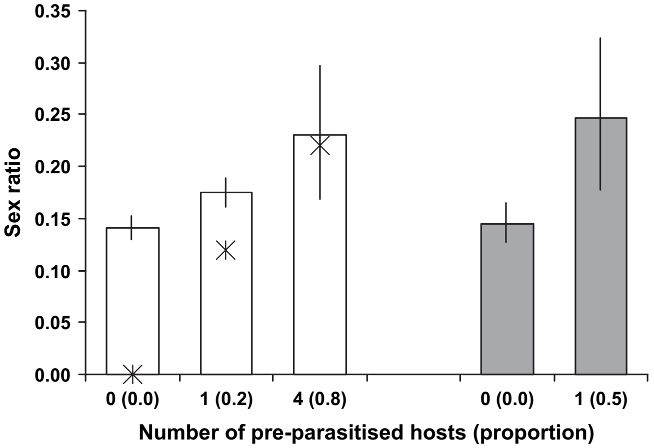

Figure 4
Sex ratios produced by females ovipositing on previously unparasitized hosts with respect to the number (and proportion) of preparasitized hosts on a five-host (open bars) or two-host (shaded bars) patch. The asterisks (*) represent the predicted sex ratios on the five-host patches from our asymmetrical LMC model, with the caveat that we here combine all offspring from the first female(s) into one first brood (see text). The parameters are the clutch sizes (one preparasitized host: $c_{11} = 22.66$ and $c_{22} = 52.21$; four preparasitized hosts: $c_{11} = 94.29$ and $c_{22} = 23.57$), the relative competitive abilities of the males from the two broods (in this experiment emergence asynchrony is 24 h, so $h_1 = 0.757$ and $h_2 = 0.192$), the sex ratio of the first brood ($x_{11} = 0.141$), and the inbreeding coefficient ($F = 0.31$ as in Figure 2). The error bars are 95% confidence intervals.

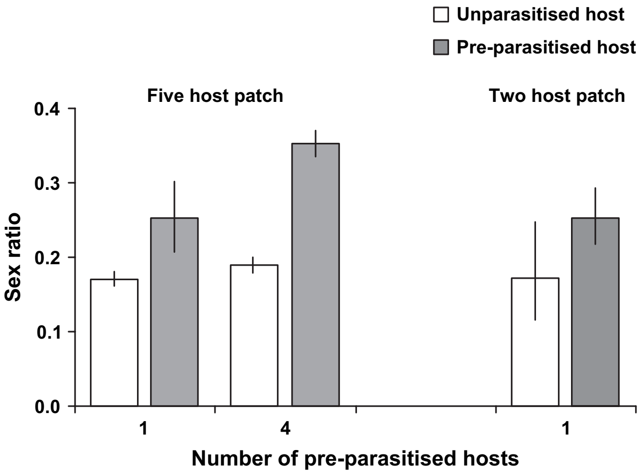

Figure 5
Sex ratios produced by females ovipositing on both unparasitized (open bars) and preparasitized hosts (shaded bars) with respect to the number of preparasitized hosts on a five-host or two-host patch. Error bars are binomial standard errors.

females in preparasitized hosts dependent on the number of unparasitized hosts also available for use on the patch (treatments B–D: $F_{2,142} = 25.47$, $p < .0001$; Figure 3c). In addition, in treatments B–D, the number of wild-type brood was strongly negatively associated with the number of red-eye brood, with females producing less offspring when there were a greater number of red-eye brood already present ($F_{1,145} = 53.71$, $p < .0001$, $R^2 = .27$, $\beta = -0.34 \pm 0.05$). This relationship did not significantly covary with treatment ($F_{2,143} = 1.83$, $p = .16$), suggesting that it is the number of eggs already present rather than the number of parasitized hosts that is important.

Sex ratio

Our main prediction is that females alter their sex allocation on unparasitized hosts with respect to how many other hosts in the patch have already been parasitized by other females. Within treatments B and C (patches with one or four preparasitized hosts), females varied as to whether they used only unparasitized hosts or a mixture of unparasitized and preparasitized hosts (see above). To test our predictions, we consider these females separately. For females that only used unparasitized hosts, there was a significant positive relationship between sex ratio and the number of preparasitized hosts on a patch, with sex ratio increasing with increasing number of preparasitized hosts as predicted (treatments A, B, and C: $F_{1,110} = 6.04$, $p = .016$; Figure 4). There was also a negative association between number of offspring and sex ratio as predicted ($F_{1,110} = 4.74$, $p = .03$). We can extend our analysis by also adding in the two two-host treatments (E and F). There was a highly significant positive relationship between proportion of hosts preparasitized and sex ratio as predicted ($F_{1,177} = 31.77$, $p < .0001$; Figure 4) and a marginally nonsignificant negative relationship between sex ratio and number of offspring ($F_{1,176} = 3.19$, $p = .07$). The significant difference between treatments E and F confirms the findings of our previous asymmetrical LMC paper.

For females that used both parasitized and unparasitized hosts, sex allocation patterns were necessarily more complex. The number of preparasitized hosts influenced sex ratio for females who oviposited on both parasitized and unparasitized hosts, with females producing less female-biased sex ratios in treatment C and also when superparasitizing (GLMMs with female as a random factor: host type $\chi^2 = 39.82$, $p < .0001$; treatment $\chi^2 = 5.42$, $p = .02$; Figure 5). The effect of superparasitizing is stronger ($R^2 = 10.7\%$ versus 1.5%), but the treatment difference is consistent when using unparasitized hosts or parasitized hosts (interaction between treatment and host type nonsignificant: $\chi^2 = 2.09$, $p = .15$). Females do therefore change their sex allocation on previously parasitized and unparasitized hosts with respect to the overall composition of the patch. We can also look at this by adding in the females who used both kinds of hosts in the two-host treatment F. The effect was dependent on the number of offspring and which host the female used (significant interactions in the minimal adequate model were treatment × number of offspring × host type: $F_{2,75} = 4.37$, $p = .02$ and treatment × host type: $F_{1,75} = 3.07$, $p = .052$), with the trend being for increased sex ratios if more hosts on the patch were preparasitized, in both unparasitized and preparasitized hosts (Figure 5). Treatment itself was nonsignificant as a main effect ($F_{2,75} = 1.19$, $p = .31$), presumably because the sex ratios in unparasitized hosts for treatments B and F were very similar. As expected, whether or not a female was superparasitizing had a strong effect on sex ratio ($F_{1,75} = 28.89$, $p < .0001$). The predicted negative relationship between sex ratio and number of offspring was marginally nonsignificant ($F_{1,75} = 3.80$, $p = .06$).

The number of hosts on a patch (five versus two) did not in itself influence sex ratio (comparing the two control treatments where all hosts were unparasitized: $F_{1,175} = 0.08$, $p = .77$). In addition, the treatments C and F (one unparasitized host with either four preparasitized hosts or one preparasitized host) also did not differ in sex ratio ($F_{1,176} = 0.15$, $p = .70$).

When females did superparasitize in the experiment, there were complicated interactions between the numbers of wild-type and red-eye offspring, making it difficult to tease apart the predicted relationships. Among the five host treatments where females did superparasitize (treatments B–D), relative clutch size and the number of red-eye offspring interacted to influence sex ratio ($F_{1,63} = 18.64$, $p < .0001$), as almost did treatment and number of (wild type) offspring ($F_{2,61} = 2.75$, $p = .07$). Taking brood numbers into account, among these superparasitizing females there was a significant treatment main effect, with higher sex ratios being produced when more of the hosts on the patch had already been parasitized ($F_{1,68} = 4.09$, $p = .02$). The predicted negative relationship between relative clutch size and sex ratio was marginally nonsignificant ($F_{1,68} = 2.95$, $p = .09$). The results are very similar if the superparasitizing females from treatment F are included.

Finally, although sample sizes were limited, we used the females in treatment C to provide comparisons between females who oviposited in both parasitized and unparasitized hosts with those who just used parasitized or unparasitized hosts. We could therefore test whether sex ratios were influenced by the pattern of host use as predicted by our theory. Using a GLMM to control for females supplying one or two data points, females produced a higher sex ratio when ovipositing on preparasitized hosts as expected ($F_{1,33} = 31.78$, $p < .0001$), but whether they oviposited on one or both types of hosts in the patch did not influence their sex ratio ($F_{1,49} = 1.62$, $p = .21$). The interaction between these two factors was also not significant ($F_{1,33} = 0.03$, $p = .86$).

DISCUSSION

It is well accepted that LMC influences sex allocation in *N. vitripennis*. We have recently developed LMC theory to take account of the variation in the level of LMC that can occur across different hosts within a patch, when different females lay eggs on a patch sequentially (Shuker et al., 2005). In addition, we have provided support for the basic prediction that females should produce a less female-biased sex ratio in unparasitized hosts if a patch contains both parasitized and unparasitized hosts (Shuker et al., 2005). Here we have shown that females adjust their offspring sex ratios in response to local variation in the extent of asymmetric LMC, demonstrating a further level of complexity in the behavior of individuals. Specifically we have shown that (1) females respond to the age of brood in already parasitized hosts on a patch (Figure 2) and (2) females respond to the number of already parasitized hosts on a patch (Figure 4).

The data from experiment 1 provided a qualitative fit to theory in that the sex ratio showed a domed relationship with the age of the first brood. However, the sex ratios did not vary across the 48- to 168-h treatments, over which we expected a gradual drop from the sex ratio produced with a 24-h brood. We can test theory quantitatively by using the clutch sizes produced in the experiment and our previous estimates of male mating success to predict how the evolutionarily stable strategy (ESS) sex ratio varies with the time between females ovipositing on a patch (Figure 2; Shuker et al., 2005). A complication here is the fact that females are constrained to produce a minimum number of sons to mate their daughters (Green et al., 1982). If we take the minimum proportion of sons to be that produced when a single female lays eggs on a patch alone (0.15), then the ESS-predicted sex ratios are all equal to or below this. This can explain why the sex ratio does not vary across the 48- to 168-h treatments but suggests that sex ratio produced in the presence of a 24-h treatment is too high. This means that either our laboratory-obtained parameter estimates are biased or that our model misses something important about how females assess their environment, such as the likelihood that another female may visit the patch at a later point (reducing the extent of LMC and favoring a more female-biased sex ratio).

Experiment 2 provided qualitative support for theory in that the sex ratio produced in an unparasitized host was positively correlated with the number of previously parasitized hosts on the patch (Figure 4). We do not have estimates of the degree of mating asynchrony between broods on patches with more than two hosts, and so testing our model quantitatively is not straightforward. However, if we consider the broods from the previous one or four females on the patch as representing just one "combined first female brood" and we use the relative competitiveness of males from the first and second broods with 24 h oviposition asynchrony as above, then we can predict the optimal sex ratio for the second female on the patch (see Figure 2 caption for parameter estimates). The observed sex ratios were not significantly different from those predicted when four hosts were preparasitized (predicted = 0.22; observed = 0.23, 95% confidence interval = 0.17–0.30) but significantly lower when one host was preparasitized (predicted = 0.12; observed = 0.17, 95% confidence interval = 0.16–0.19). However, this latter case can again be explained by the need to produce enough males.

The results of both our experiments suggest that allowing for the fact that females are constrained to produce a minimum number of males is crucial for quantitative tests of theory. There are a number of theoretical studies investigating this constraint (Green et al., 1982; Heimpel, 1994; Nagelkerke and Hardy, 1994; West and Herre, 1998; West et al., 1997). Qualitative support for its general importance has come from studies on parasitoid wasps showing a negative correlation between the sex ratio and brood size both within and across species (Griffiths and Godfray, 1988; Hardy and Cook, 1995; Hardy and Mayhew, 1998; Hardy et al., 1998; Herre et al., 1997; Mayhew and Godfray, 1997; Morgan and Cook, 1994). This negative correlation is expected because producing a minimum number of males has a larger effect on the sex ratio at smaller brood sizes. However, in most cases, we are unable to predict the actual quantitative proportion of males. For example, in *N. vitripennis*, why should it be 15% and not 10% or 5% males? In particular, females tend to produce more males than we would expect, except with very small brood sizes where only one male is produced. Solving this problem will require detailed studies of mortality rates and insemination capacity (Hardy et al., 1998, 2000; West et al., 1998). More generally, the need to produce enough males has also attracted much attention as an important constraint that could explain sex ratio variation in malaria and related protozoan parasites (Gardner et al., 2003; Reece et al., 2005; West et al., 2001, 2002).

To conclude, our experiments illustrate three general points about sex ratio evolution. First, they emphasize the importance of patch complexity. Studies typically assume that patches are homogenous. However, wasps and their hosts may visit patches over long periods of time, leading to patches with asynchronous emergence and wide variation in the number of parasitized hosts. Our results confirm that it matters which hosts within a patch a female uses and when. This will be especially important in interpreting field data, which are increasingly suggesting such complexities (Frank, 1985b; Molbo and Parker, 1996; Werren, 1983; Zavodna et al., 2005; Koevoets T, Burton M, Sykes EM, Shuker DM, West SA, and Beukeboom LW, unpublished data). Second, they demonstrate the importance of examining how sex ratio behavior coevolves with other traits (Frank, 1990; Greeff, 1997; Nagelkerke, 1994; West et al., 1999). The host choice and clutch size behavior of a female will determine the extent of asymmetrical LMC her offspring will encounter and hence the favored sex ratio strategy. Third, they emphasize the need to determine the mechanisms by which females obtain the appropriate information about their environment (Boomsma et al., 2003; Shuker and West, 2004). We have shown that the behavior of females can depend on a complex interaction of factors such as the number of parasitized and unparasitized hosts in a patch, the relative clutch sizes previously laid on hosts, as well as when wasps will emerge from different hosts. This raises a number of other questions, such as to what extent the same or different cues are used for different behaviors (King and Skinner, 1991) and to what extent does lack of perfect information constrain the behavior of individuals (Shuker and West, 2004).

We are very grateful to Aleta Graham and Alison Duncan for all their help in the laboratory. Loeske Kruuk kindly provided advice on the use of GLMMs. Ian Owens, Peter Mayhew, and one anonymous reviewer made very useful comments, which helped us improve the manuscript. We were supported by the Biotechnology and Biological Sciences Research Council. Natural Environment Research Council, and the Royal Society. The experiments carried out complied with current Scottish and U.K. laws.

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