Facultative Sex Ratio Adjustment in Natural Populations of Wasps: Cues of Local Mate Competition and the Precision of Adaptation

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ABSTRACT: Sex ratio theory offers excellent opportunities to examine the extent to which individuals adaptively adjust their behavior in response to local conditions. Hamilton's theory of local mate competition, which predicts female-biased sex ratios in structured populations, has been extended in numerous directions to predict individual behavior in response to factors such as relative fecundity, time of oviposition, and relatedness between cofoundresses and between mates. These extended models assume that foundresses use different sources of information, and they have generally been untested or have only been tested in the laboratory. We use microsatellite markers to describe the wild oviposition behavior of individual foundresses in natural populations of the parasitoid wasp *Nasonia vitripennis,* and we use the data collected to test these various models. The offspring sex ratio produced by a foundress on a particular host reflected the number of eggs that were laid on that host relative to the number of eggs that were laid on that host by other foundresses. In contrast, the offspring sex ratio was not directly influenced by other potentially important factors, such as the number of foundresses laying eggs on that patch, relative fecundity at the patch level, or relatedness to either a mate or other foundresses on the patch.

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Sex ratio theory has provided excellent opportunities to examine the precision of adaptation (Charnov 1982; Herre 1987; Hardy 2002; West and Sheldon 2002; Boomsma et al. 2003; Shuker and West 2004). One of the most productive areas in this regard has been Hamilton's theory of local mate competition (LMC), which explains why female-biased sex ratios are favored in structured populations, where mating occurs before females disperse (Hamilton 1967). Specifically, if n diploid foundresses lay eggs on a patch, then the evolutionarily stable (ES) sex ratio (r^*; the proportion of males) is defined as $r^* = (n - 1)/2n$ (Hamilton 1967). This equation predicts that a decrease in the number of foundresses will result in a more female-biased sex ratio and that an ever-increasing number of foundresses will cause the sex ratio to become less female biased, with an asymptote toward 0.5. One way of conceptualizing this is that a female bias is favored because it reduces competition between sons (brothers) and increases the number of mates for sons (Taylor 1981). An additional bias is favored in haplodiploid species because inbreeding makes females relatively more related to their daughters than to their sons (Frank 1985b; Herre 1985). There is extensive empirical support for the basic predictions of LMC theory: foundresses of numerous species have been shown to adjust their offspring sex ratios in response to the number of foundresses laying eggs on a patch (West et al. 2005).

Extensions of LMC theory have suggested that the pattern of sex ratio adjustment should vary depending on how much information foundresses are able to process about the environment. Hamilton's original prediction was based on a number of simplifying assumptions, such as foundresses contributing the same number of offspring to each patch and random mating within the patch (Ham-

ilton 1967). These assumptions implicitly constrain what information foundresses are thought to use. When these assumptions are relaxed, offspring sex ratios are predicted to vary within the patch, between individuals, and over time and space (Suzuki and Iwasa 1980; Werren 1980; Frank 1985b, 1987; Yamaguchi 1985; Stubblefield and Seger 1990; Taylor and Crespi 1994; Abe et al. 2003; Reece et al. 2004; Shuker et al. 2005). For example, Werren (1980) demonstrated that, when two foundresses lay eggs sequentially on the same host, the sex ratio produced by the second foundress to lay eggs on the host should be negatively correlated with the relative size of her clutch (i.e., clutch laid by the second foundress divided by the clutch size of the first foundress). This is because, when a foundress produces a lower proportion of offspring, her offspring will experience less LMC (i.e., less competition between brothers). It has since been demonstrated that the same qualitative prediction—to produce a less female-biased sex ratio (or even to produce a male-biased sex ratio)—arises in a range of other models that contain simultaneous oviposition (Yamaguchi 1985; Frank 1987; Stubblefield and Seger 1990). Table 1 summarizes these

models and identifies the variables that are predicted to influence sex ratio. Although these models have been tested several times in the laboratory (see below), there has been a conspicuous absence of field tests that examine what information foundresses actually use when varying their sex ratio under LMC. This is largely because of the technical difficulties of recording oviposition behavior in the field.

Herein we address the problem of recording oviposition behavior in the field by using microsatellite markers to trace the field behavior of individual foundress females of the parasitic wasp *Nasonia vitripennis. Nasonia vitripennis* is an ideal organism for such a study because it is known from both laboratory studies and field studies that the females adjust their sex ratios in response to the basic tenets of LMC (Werren 1983; Orzack et al. 1991; Molbo and Parker 1996; Shuker and West 2004; Grillenberger et al. 2008). *Nasonia vitripennis* has also been extremely useful in testing the more complex LMC models, but thus far these studies have been restricted to the laboratory (Werren 1980; Orzack and Parker 1986, 1990; Flanagan et al. 1998; Reece et al. 2004; Shuker et al. 2004a, 2004b,

Table 1: Models of sex allocation under local mate competition (LMC), in terms of the information foundresses are predicted to use and the variables associated with the models in our empirical study

Model name	Predicted information use	Empirical variables associated with the model
Hamilton (Hamilton 1967, 1979)	Patch foundress number, $s^* = (2n - 1)(n - 1)/n(4n - 1)$	Patch foundress number
Stubblefield and Seger model 1 (SandSI; Stubblefield and Seger 1990)	Knowledge of own fecundity, no knowledge of cofoundress fecundity ("imperfect knowledge")	Focal foundress fecundity (defined at the level of the host or patch)[a]
Stubblefield and Seger model 2 (SandSII; Frank 1985b; Herre 1985; Stubblefield and Seger 1990)	Knowledge of own fecundity and cofoundress fecundity ("perfect knowledge")	Focal foundress and cofoundress fecundity (defined at the level of the host or patch)[a]
Werren (host; Suzuki and Iwasa 1980; Werren 1980)[b]	Relative clutch size (focal foundress relative to cofoundresses) on a given host	Relative clutch size of focal foundress on a host (as difference in clutch sizes between focal and cofoundresses)
Werren (patch; Suzuki and Iwasa 1980; Werren 1980)[b]	Relative clutch size (focal foundress relative to cofoundresses) across the patch	Relative clutch size of focal foundress on a patch (as difference in clutch sizes between focal and cofoundresses)
Asymmetrical LMC (Nunney and Luck 1988; Shuker et al. 2005)	Knowledge of own and cofoundress fecundities across both individual hosts and the patch as a whole	Focal foundress and cofoundress fecundities across hosts and patch
Greeff (Greeff 1996; Reece et al. 2004)	Relatedness to mating partner and foundress number	Relatedness to mating partner and foundress number
Frank (Frank 1985b, 1998; Taylor and Crespi 1994; Shuker et al. 2004a)	Relatedness to cofoundresses and foundress number	Relatedness to cofoundresses and foundress number

[a] Originally defined at the level of the patch, but if mating is increasingly nonrandom within a patch (Shuker et al. 2005), then each host effectively becomes a patch.

[b] The original Werren model (1980) is for sequential oviposition by two foundresses, with the focal foundress being the second foundress. The predicted sex ratio is influenced by the primary sex ratio, the population inbreeding coefficient, and relative clutch size. We use it here in a general sense to consider sex allocation on the basis of relative clutch size.

2006, 2007). In our study we used the power and resolution of molecular techniques to test these extensions to LMC theory in the wild. Specifically, we (1) tested to what extent foundresses adjust their sex ratio in response to predicted environmental parameters (table 1) and (2) determined which models of LMC best approximate sex allocation in the wild. By genotyping >3,500 offspring at four microsatellite loci, we were able to reconstruct the parental genotypes and thus determine the sex ratios produced by 49 foundresses in 350 broods across 18 natural patches. Our results provide the first detailed analysis of individual sex allocation under LMC in the wild.

Material and Methods

Study Organism

Nasonia vitripennis is a gregarious parasitic wasp, with females laying clutches of eggs on a range of large Diptera pupae such as *Calliphoridae* and *Sarcophagidae* (Whiting 1967). The species is ectoparasitic, with the eggs being laid between the pupa and puparium wall and the adults emerging from the host puparium to mate. Males are brachypterous and unable to fly and are typically the first to emerge. They then mate with the emerging females. When multiple hosts on a patch are parasitized, mating is typically nonrandom, with males and females from the same host more likely to mate with one another (Van den Assem et al. 1980*a*, 1980*b*; Shuker et al. 2005). Females are fully winged and disperse away from the host. The mating system typifies that which was assumed by LMC, and *N. vitripennis* has long been an outstanding model organism for the study of sex ratios.

Sampling

We used two field sites: one in Hoge Veluwe National Park, Netherlands (HV), and one at a field site near Schlüchtern, Hessen, Germany (Schl). A companion article by Grillenberger et al. (2008) provides full details of the sampling and subsequent genetic analysis involved in this study. That article also describes the patterns of oviposition on the patches and the population genetics of the two study populations. Briefly, we collected *N. vitripennis* broods in June 2004 from bird nest boxes ("patches") either by searching for parasitized host puparia (*Calliphora vicina*; HV only) or by leaving unparasitized host puparia as baits at patches (patch size, 25 hosts [both HV and Schl]). The HV collection comprises 10 patches, nine of which were "natural" collections and one that was a successfully baited sample. The Schl collection comprises eight successfully baited patches. All fly puparia were collected and incubated individually at 20°C.

Each day we brought the incubated hosts into the daylight for at least 30 min before anesthetizing any emerged individuals with CO_2 and storing them for molecular analysis. We checked for any unemerged individuals by opening the fly puparia 1 month after the last emergence from that host. We recorded the origin of every individual in terms of field site, patch, and host. The full details of the number of parasitized hosts and the individual broods are given in table A1. Throughout the study, we considered the number of emerged offspring to equal the number of eggs laid by foundresses (clutch size), thereby assuming negligible larval mortality. Although this has been shown to be the case under laboratory conditions (Werren 1984), we do not know the impact of larval mortality in the wild.

Molecular Genetic Analysis

We extracted whole genomic DNA from individual wasps using either a standard high salt-chloroform protocol (Maniatis et al. 1982) or Chelex 100 (Bio-Rad, Hercules, CA). For genotyping, we used four polymorphic, dinucleotide repeat microsatellites (Nv-22, Nv-23, Nv-41, and Nv-46). Pietsch et al. (2004) originally developed Nv-22 and Nv-23, but they were redesigned for this study (table A2). We separated polymerase chain reaction products by fragment length using an AB 3730 DNA analyzer or ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA) and analyzed the products using either GeneMapper 4.0 or GeneScan 3.1 (Applied Biosystems).

We sexed all individuals according to external morphology before DNA extraction was performed; damaged individuals were checked by their heterozygosity (e.g., heterozygotes must be female). Parentage was assigned according to Mendelian rules of inheritance under haplodiploidy. The genotypes of the foundresses that oviposited on each host were reconstructed from the genotypic data of the offspring. Each patch was resolved with the minimum number of foundresses required to explain the offspring. For the analysis presented above, two patches were excluded. In the first case, a solitary foundress oviposited on one host in the patch, producing only sons. This foundress may have, therefore, been a virgin and thus unable to produce daughters (a "constrained" female). We also excluded a nest box that contained 16 parasitized hosts and up to seven foundresses. In this case, assigning offspring to a foundress was difficult because some of the foundresses and their respective mates appeared to be very closely related. This meant that numerous offspring had multiple possible mothers. Inclusion of these two patches does not qualitatively alter the results presented. The following analysis, therefore, considers 16 patches containing 324 clutches from 47 foundresses laid on 222 hosts. These clutches produced 3,027 genotyped offspring that were assigned to a foundress.

We calculated the average relatedness between all foundresses on each patch and between each foundress and her mate(s), following the principles of Queller and Goodnight (1989). We used the Relatedness 5.0.8 program (Goodnight and Queller 2001) to generate relatedness values on a scale from -1.0 to 1.0. We treated the HV and Schl samples as two distinct populations, and the estimate of the population allele frequencies was bias corrected for each foundress by excluding both her and her mate. We excluded the cases of a single foundress parasitizing a patch from the analysis of patch relatedness.

Statistical Analyses

We performed two analyses. First, we tested explanatory variables at the host and patch level. For the second analysis, we tested specific statistical models appropriate for different models of LMC. For the first analysis, the explanatory variables were patch foundress number, host foundress number, difference in fecundity of focal foundress versus other foundresses on the host (or on the patch), focal foundress fecundity, patch size (defined as total number of hosts), numbers of parasitized hosts (parasitized by the focal foundress and by all foundresses on the patch), proportion of the hosts parasitized, relatedness between foundresses on a patch (if appropriate—see above), and relatedness of a foundress to her mate.

The difference in fecundity between a focal foundress and the other foundresses on the host (or patch) was calculated by subtracting the number of offspring produced by other foundresses from the number produced by the focal foundress. This allowed us to consider a form of relative clutch size, a potentially important variable (Werren 1980), usually calculated as focal foundress clutch size divided by nonfocal foundress clutch size. However, this definition cannot be applied to foundresses that have oviposited by themselves, thereby necessitating the use of difference in fecundity. When we specifically considered just those hosts with more than one foundress (i.e., superparasitism), the more usual relative clutch size of the focal foundress was used.

For one patch, the total number of hosts (parasitized plus unparasitized) was not known because of a recording error. Therefore, the fixed effects "patch size" and "proportion of parasitized hosts" were only tested on the subset of 15 patches with this information. One potentially informative variable that we were unable to measure is laying order (i.e., the sequence in which particular foundresses contributed eggs to a host or patch). Emergence times of wasps do not provide reliable oviposition order data because, in *N. vitripennis*, superparasitism can lead to synchronized development of the different broods within a host (Werren 1980). Although relative clutch size is a pos-

sible proxy of laying order (because, in gregarious parasitoids, superparasitizing foundresses typically produce a relatively smaller clutch size; e.g., Godfray 1994), we could not be sure this would always be the case. This problem with laying order is a necessary constraint of this kind of study (also see "Discussion").

Sex ratios are best modeled within a generalized linear modeling framework that assumes binomially distributed errors and that has a logit link function (Wilson and Hardy 2002). Because foundresses could contribute multiple clutches, for the first analysis we used a generalized linear mixed modeling approach (GLMM) and included foundresses' identities as a random effect to take these multiple observations into account. However, GLMMs are still an area of active research, and current tractable estimation methods do not generate true likelihoods; rather, they use approximations to complete the integration. We used restricted penalized quasilikelihood (REPQL) as provided by the glme function in the Correlated Data Library in S-Plus 7 (Pinheiro and Chao 2005). Other methods for binomially distributed data (Laplacian and adaptive Gaussian quadrature methods) force the dispersion parameter to equal 1 (i.e., they assume true binomial variance), but our data were slightly overdispersed (dispersion parameter, 1.555). The fixed effects were tested using marginal *t*-tests with approximate degrees of freedom (Pinheiro and Chao 2005). Models were simplified by removing the least significant terms in turn to generate the minimum adequate model. For completeness, given that several of the explanatory variables associated with different models of LMC are likely to be correlated with each other, we also tested variables alone in individual models.

For our second analysis, because GLMMs do not yield true likelihoods, we were unable to compare different models using techniques such as likelihood ratio tests or Akaïke Information Criterion (AIC). To test how well different models of sex allocation predict wild sex ratios, we therefore fitted specific models in turn to the sex ratio data using a maximum likelihood mixed effects framework (table 1). Model fit was examined by way of AIC and the models were compared. All statistics were performed using S-Plus 7 (Pinheiro and Chao 2005). Means ± SE are presented, with asymmetric binomial SEs for sex ratio.

Results

Descriptive Statistics

The overall sex ratio across the 16 patches was extremely female biased (mean ± SE, 0.200 ± 0.007). The number of foundresses laying eggs on patches ranged from one to seven, and on individual hosts, from one to four. The average clutch size per host per foundress was 9.34 ± 0.40 ($n = 324$ clutches). For those hosts where only one

foundress laid eggs, average clutch size ± SE was 11.56 ± 0.64; in those hosts where multiple foundresses laid eggs, it was only 7.74 ± 0.48. Sex ratio did not differ among populations (removed from the full model: $t = 0.02$, df $= 36$, $P = .98$), and thus the analysis below considers both populations together. Sex ratios varied significantly among foundresses (among-foundress variance component, 1.225; 95% confidence interval, 0.654–2.292). The average relatedness between foundresses on a patch varied from −0.46 to 0.28, with mean values ± SE of 0.09 ± 0.04 for HV and −0.05 ± 0.05 for Schl. The average relatedness of a foundress to her mate(s) suggested appreciable levels of sibmating: the mean relatedness value ± SE was 0.32 ± 0.04 for HV ($n = 27$) and 0.22 ± 0.02 for Schl ($n = 19$); values ranged from −0.43 to 0.82.

Sex Ratios

Sex ratios varied with the difference in clutch sizes that foundresses produced on a host, with foundresses producing more female-biased sex ratios when they laid relatively more eggs on a host ($t = 8.23$, df $= 282$, $P < .0001$; fig. 1). The quadratic term was not significant ($t = 1.18$, df $= 278$, $P = .24$). When difference in clutch size at the level of the host was fitted in the model, no other factors were statistically significant (table 2).

The relative number of offspring that a foundress produced on a host or a patch was negatively correlated with the number of foundresses laying eggs on that host or patch (host foundress number and difference in fecundity on that host, $r = -0.66$, df $= 322$; patch foundress number and difference in fecundity on that patch, $r = -0.22$, df $= 322$; $P < .0001$ for both). When difference in clutch size was not included in the model, the sex ratio was positively

correlated with both the number of foundresses laying eggs on a host ($t = 6.34$, df $= 282$; $P < .0001$; fig. 2) and the number of foundresses laying eggs on a patch ($t = 2.74$, df $= 282$, $P = .007$; fig. 2). There was also a weak negative correlation between sex ratio and the total number of offspring a foundress contributes to a patch when these variables were fitted alone ($t = 2.05$, df $= 282$, $P = .04$).

The above data set considers all foundresses together and combines different patterns of patch and host use. It is also useful to consider some specific cases. In the simplest case, an individual female was the only foundress on a patch ($n = 4$). With no cues indicating reduced LMC, sex ratios were highly female biased (sex ratio, 0.084; lower SE, 0.016; upper SE, 0.019) and independent of clutch size (per host: $t = 0.59$, df $= 12$, $P = .57$; per patch: $t = 0.12$, df $= 2$, $P = .92$), although, of course, the sample was very small. Alternatively, other foundresses used one host individually but shared the patch as a whole with other foundresses ($n = 27$). Foundresses did not shift their sex ratios on these hosts in response to the characteristics of the rest of the patch. Their sex ratios were not correlated with patch foundress number ($t = 1.24$, df $= 23$, $P = .23$), clutch size on the host ($t = 1.05$, df $= 91$, $P = .30$), total fecundity of the focal foundress on the patch ($t = 1.01$, df $= 23$, $P = .32$), or the difference in fecundity between the focal foundress and all the other foundresses across the patch ($t = 0.82$, df $= 23$, $P = .42$). Finally, two or more foundresses shared particular hosts (superparasitism; $n = 35$ foundresses). Sex ratios were highly significantly correlated with relative clutch size (defined here as focal foundress clutch size divided by nonfocal foundress clutch size; see "Material and Methods"), with sex ratios declining with increasing relative clutch size as expected according to theory (fig. 3). Both
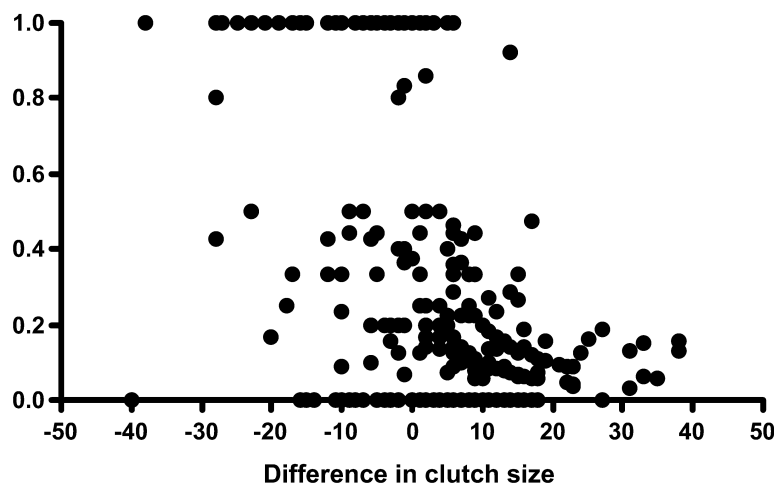

Figure 1: Sex ratios are negatively correlated with the difference in clutch size between foundresses ovipositing on a host.

Table 2: Analysis of sex ratio variation

Fixed effect	Fitted together		Fitted alone	
	t (df)	P	t (df)	P
Patch foundress number	1.28 (279)	.20	2.74 (282)	.007
Host foundress number	1.06 (277)	.29	6.34 (282)	<.0001
Relative fecundity per patch	.65 (274)	.52	1.64 (281)	.10
Quadratic term	1.64 (281)	.10	.66 (281)	.51
Relative fecundity per host	8.23 (282)	<.0001	8.09 (281)	<.0001
Quadratic term	1.18 (278)	.24	1.57 (281)	.12
Focal foundress patch fecundity	.55 (276)	.59	2.05 (282)	.04
Number of hosts used by focal foundress	.96 (275)	.34	.99 (282)	.32
Total number of hosts used on a patch	1.46 (280)	.15	.01 (282)	.99
Patch size	1.43 (34)	.16	.03 (34)	.98
Proportion of hosts used	1.13 (274)	.26	.09 (275)	.93
Patch relatedness	.92 (268)	.36	.59 (269)	.56
Mate relatedness	.34 (37)	.74	.02 (37)	.99

Note: Fixed effects were either (1) tested by model simplification with the all terms fitted together in the full model, with the least significant terms removed in turn and with significance tested after the fitting of any other significant effects; or (2) fitted alone in a model (apart from the relative fecundities that are fitted with their respective quadratic terms). The t values are marginal t tests presented with approximate degrees of freedom.

relative clutch size and its quadratic term were highly significant ($t = 4.47$, df $= 151$, $P < .0001$, and $t = 3.81$, df $= 151$, $P < .0001$, respectively). The theoretical prediction for sex allocation under superparasitism according to Werren (1980; adjusted for haplodiploidy) includes the sex ratio of eggs already present on a host and the inbreeding coefficient. Using the sex ratio produced by foundresses when ovipositing on a patch alone and the population inbreeding coefficient $F_{IT} = 0.197$ (Grillenberger et al. 2008), the Werren model (1980; adjusted for haplodiploidy) also predicts a highly significant proportion of the variance in sex ratio ($t = 4.04$, df $= 152$, $P < .0001$; fig. 3; table 3).

Testing LMC Models

Models of sex allocation under LMC form a hierarchy, with more complicated models assuming that foundresses use increasingly sophisticated information to estimate the level of LMC (table 1). By including the appropriate variables for each model, we can assume that foundresses process increasing amounts of information about the patch. Doing so explains increasing amounts of variation observed in sex ratios in the field (table 3). The best-fitting models suggest that complete knowledge of the clutch sizes of the foundresses on a given host, either in absolute terms or as the difference between them, is crucial for explaining the sex ratio. The best-fitting model of all is the "Werren (host)" model (1980). This also corresponds to the empirically derived minimal model from the above analysis, which contains the difference in fecundity on a host. For the specific case of superparasitism, the empirically derived

model above (relative clutch size and its quadratic term) fits the data marginally better than a fully parameterized version of the Werren model (1980).

Discussion

We used microsatellite markers to determine the sex ratio behavior in the field of individual *Nasonia vitripennis* foundresses. We found that the only significant variable was the relative clutch size laid on a host: foundresses produced a less female-biased sex ratio when they laid relatively fewer eggs on a host (fig. 1). When this effect was included in the model, no other factors were significant (table 2). We also tested the extent to which different LMC models could explain variation in sex ratio. We found that, whereas models constructed purely on the basis of the number of foundresses laying eggs on a patch (Hamilton 1967) or the relative fecundity on a patch (Stubblefield and Seger 1990) were statistically significant, they did not fit the data as well as models formed on the basis of relative fecundity at the host level (Suzuki and Iwasa 1980; Werren 1980; Shuker et al. 2005; table 3).

Our results suggest that foundresses are adjusting their offspring sex ratios in response to variation in the extent of LMC and that the primary cue on which they are basing their behavior is the relative number of eggs that they are ovipositing on each host. In contrast, they do not appear to be using information about the total number of foundresses on a patch or the relative fecundity of different foundresses on a patch. This result agrees with a recent laboratory experiment in which foundresses were shown to lay less female-biased sex ratios when cofoundresses
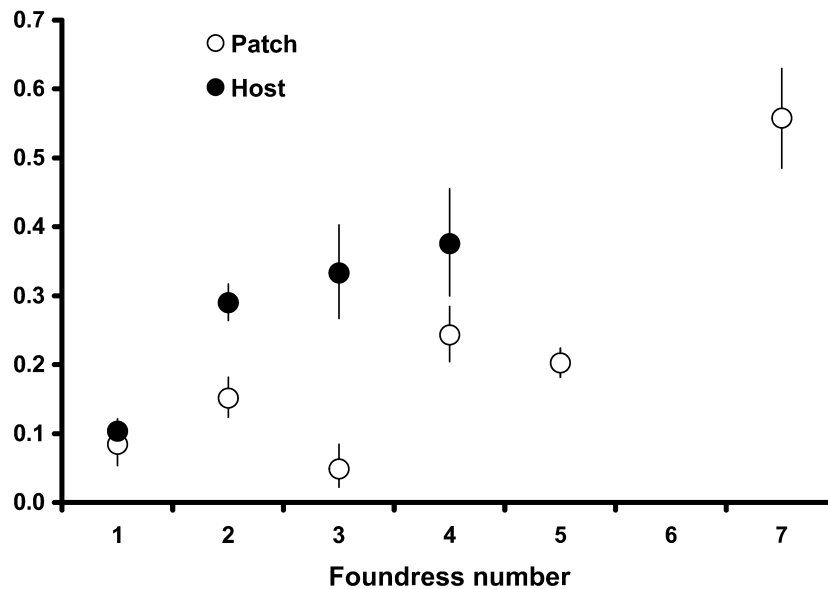
Figure 2: Sex ratios vary with the number of foundresses using the patch (*open circles*) or a particular host (*filled circles*). Error bars are 95% binomial confidence intervals.

were present; rather, the primary cue to do so was the eggs laid by those other foundresses and not the presence of the foundresses themselves (Shuker and West 2004). We suggest that the explanation for these results is that foundresses are responding to the cues that are reliable indicators of the extent of LMC that their offspring will experience under natural conditions. Foundresses appear to be able to assess with relative ease whether a host has been previously parasitized (Werren 1984; King et al. 1995; Shuker et al. 2005, 2006), and a higher proportion of previously parasitized hosts should correlate with less LMC. In contrast, foundresses may not be able to directly assess the number of foundresses that are laying eggs on that patch, especially if these foundresses visit the patch sequentially. Relative clutch size is also likely to be associated with laying order of foundresses (Godfray 1994), a factor we could not specifically test given the difficulties of accurately resolving the visitation order because of the synchronization of development in multifoundress broods (see "Material and Methods").

Another potentially important factor is that mating will often not be random within the whole patch, as assumed by most LMC models (Shuker et al. 2005, 2006, 2007). Understanding the scale at which mating occurs is, therefore, crucial. Laboratory experiments have shown that even when wasps emerge at very similar times from hosts that are next to each other, they are more likely to mate (albeit not exclusively) with individuals that developed in their own hosts (Van den Assem et al. 1980*a*, 1980*b*; Shuker et al. 2005). In nature, this effect will be increased because

hosts can be spatially separated and emergence times can be very spread out, as they were for our HV population (emergence times for the Schl population were not recorded), where the mean duration ± SE of emergence from the first to the last individual in a patch was 9.00 ± 2.36 days. At times, the difference in emergence time between hosts from the same patch was as large as 18 days, which is considerably longer than the mean life span of approximately 9 days for sexually active males in the laboratory (Burton-Chellew et al. 2007). This means that the level of LMC actually experienced by wasps may differ from that expected by observers when considering the whole patch, and that wasps from different broods on the same patch may experience different levels of LMC (asymmetrical LMC; Shuker et al. 2005). Consequently, whether a host has been previously parasitized and the relative number of eggs that a foundress lays on it may be more reliable indicators of the level of LMC that the offspring from a host will actually experience. Characteristics of the patch as a whole should not be so important. The importance of this in other species will depend on natural history details; for example, emergence and mating may be staggered in many parasitoid wasps that attack clumps of hosts (Godfray 1994; West et al. 2005), whereas the relatively synchronous oviposition and emergence of fig wasps (Hamilton 1979; Frank 1985*a*, 1985*b*; Herre 1985, 1987) should lead to relatively random mating within the patch.

What information do foundresses actually use to produce our observed negative correlation between offspring
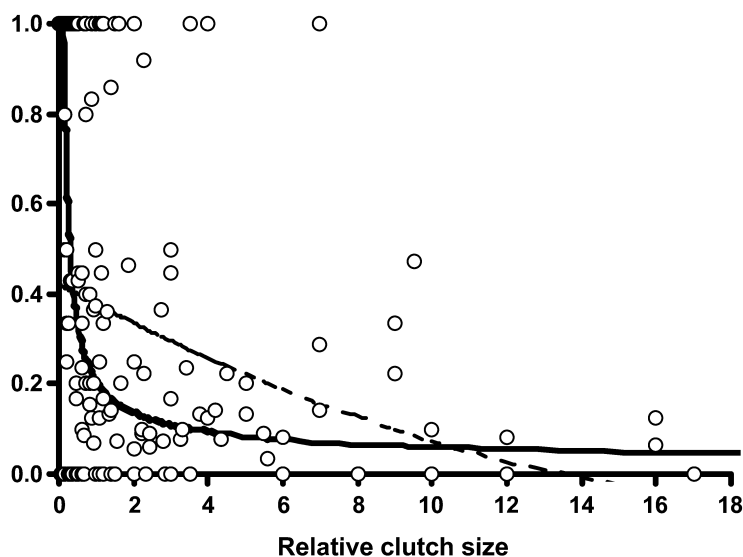
Figure 3: Sex ratios vary with relative clutch size when two or more foundresses lay eggs on the same host (superparasitism). The dashed line is the relationship between sex ratio and relative clutch size (RCS) obtained from the analysis: sex ratio $\sim (0.4211 - 0.0448) \times$ RCS $+ 0.0010 \times$ RCS^2. The solid line is the prediction from Werren (1980) adjusted for haplodiploidy. For clarity, the largest relative clutch size has been omitted from the figure (RCS, 39.0; sex ratio, 0.154).

sex ratio and the relative clutch size that a foundress lays on a host (figs. 1, 3)? Foundresses may respond to their own fecundity, regardless of whether the host has been previously parasitized or the number of previously laid eggs on the host (Werren 1980, 1984; Orzack and Parker 1990). Support for the idea that foundresses are responding to previous parasitism and the number of eggs laid previously is provided by the fact that there is (1) no correlation between absolute clutch size and sex ratio when foundresses lay eggs on a host alone (regardless of whether they share any of the other hosts on the patch; table 2) and (2) a poorer fit to the data with a focal foundress's own fecundity when compared with a focal foundress's fecundity plus other foundresses' fecundities (table 2). In addition, previous experiments have shown that foundresses are less likely to oviposit on (and lay fewer numbers of eggs on) parasitized hosts that have had a greater numbers of eggs previously laid on them (Shuker et al. 2005). As highlighted above, our analyses of the field data will also have underestimated the ability of individuals to assess the number of eggs previously laid on a host because, in superparasitized hosts, we do not know the order in which foundresses laid eggs. Consequently, the first foundresses to visit each host are also included in our analyses, despite the fact that they can have no knowledge of the number of eggs that will be laid later on the host. This limitation of a natural data set may also explain why we did not find support for the experimentally observed pattern that the sex ratio laid on a host is influenced by the extent to which other hosts on the patch have been previously parasitized (Shuker et al. 2005). Clearly there is scope for trying to bring experimental approaches to studies in the field—for example, by adding hosts to patches with known parasitism histories to separate out the effects of laying order and relative clutch size.

Further complications include the fact that foundresses do not superparasitize hosts that have been parasitized more than 48 h earlier (Werren 1984; Shuker et al. 2006) and that, as discussed above, parasitization and emergence can be relatively spread out on natural patches. In addition, foundresses may also be sperm limited and thus constrained to produce fewer daughters than they would otherwise. One foundress, excluded from the analysis presented here, produced only male offspring, which could have been the result of virginity or sperm depletion (as well as a response to relative clutch size; Werren 1980). Although a single mating of *N. vitripennis* usually provides a female with sufficient sperm to fertilize several hundred eggs, males that have recently mated with 50 or more foundresses do produce smaller ejaculates (or fail to inseminate successfully; Barrass 1961). However, in our data set, only four of the 136 clutches that were laid singly on hosts had sex ratios in excess of 0.4 (none of which exceeded 0.5). Sperm limitation, therefore, seems unlikely to be common. We also observed some broods with sex ratios of 0; these are expected to occur fairly frequently with sex ratio optimization and small broods (e.g., Heimpel 1994; West et al. 1997).

Table 3: Testing models of sex allocation that assume different sources of information for estimating the level of local mate competition (LMC) experienced by offspring for all foundresses and for only those foundresses sharing hosts (superparasitism)

Model	AIC	Log-lik	Residual	% Decrease
All foundresses:				
Random effect only	221.08	−107.54	.3111	
Hamilton	217.54	−104.77	.3100	.35
S and S I (patch)	216.18	−104.09	.3099	.39
S and S II (patch)	211.46	−100.73	.3094	.55
S and S I (host)	180.08	−86.04	.2901	6.75
S and S II (host)	**151.70**	**−70.85**	**.2800**	**10.00**
Werren (host)	**149.96**	**−70.98**	**.2804**	**9.87**
Werren (patch)	213.59	−102.80	.3098	.42
Asymmetric LMC	**153.27**	**−69.63**	**.2799**	**10.03**
Greeff	216.53	−103.27	.3091	.64
Frank	208.56	−99.28	.3106	.16
Superparasitism:				
Werren[a]	175.14	−83.57	.3425	4.38[b]
Empirical model[c]	**171.52**	**−80.76**	**.3405**	**4.94**

Note: Mixed effect models were fitted by maximum likelihood, with foundress as a random effect. Each model was fitted in turn. Models in bold represent the better-fitting models. The model Werren (host) also represents the minimal model from our empirical analysis. For full details of the models, see table 1. Model fit is described in terms of Akaike Information Criterion (AIC), log-likelihood of the model (Log-lik), residual deviance of the model (Residual), and percent decrease in residual deviance compared with the model using just the random effect (% Decrease).

[a] The specific version of the Werren (1980) model adjusted for haplodiploidy (Suzuki and Iwasa 1980; Greeff 2002) and parameterized using the single foundress sex ratio, relative clutch sizes, and inbreeding coefficient from this article and Grillenberger et al. (2008).

[b] The residual deviance after fitting the random effect only is 0.3582.

[c] Contains the variables relative clutch size and (relative clutch size)^2.

Our analyses support the results from laboratory studies of *N. vitripennis* and other species, that foundresses do not adjust their sex ratio in response to their relatedness to their mates or the other foundresses on the patch (Frank 1985*b*, 1998; Taylor and Crespi 1994; Greeff 1996; Reece et al. 2004). Foundresses are predicted to lay a more female-biased sex ratio when mated to more closely related individuals, because then they will be relatively more related to their daughters than to their sons (Frank 1985*b*; Herre 1985; Greeff 1996; Reece et al. 2004). Foundresses are also predicted to lay a more female-biased sex ratio when ovipositing with more closely related foundresses, because this will increase the relatedness between the offspring developing on the patch and thus increase the extent of LMC (Frank 1985*b*, 1986; Taylor and Crespi 1994). While it could be argued that selection for an effect with relatedness to other foundresses may be weak because relatives rarely oviposit on the same patch and because mating between related offspring emerging from different hosts may also

be limited given their distribution in space or time, there is appreciable variation in relatedness to mates because mating between both siblings and nonsiblings is common. However, such sex ratio adjustment would require reliable cues for kin recognition, and theory suggests that sufficient variability in the cues is unlikely to be maintained (Reece et al. 2004). The reason for this is that more common alleles would be recognized more often and would indicate a higher relatedness, and thus they would be under the influence of positive selection. Less common alleles would therefore be eliminated, along with the variability that is required for kin discrimination (Crozier 1986; Rousset and Roze 2007).

Conclusion

Our results show that, for species that are shown to fit simple models of LMC (West et al. 2005), techniques that allow the testing of more specific models in the wild can tell us a great deal about what limits adaptive behavior. Our results also emphasize two general points about the extent to which we should expect data to fit theory. First, the ability of individuals to adjust their behavior in response to environmental conditions depends on the cues that they can use and the reliability of those cues (West and Sheldon 2002; Boomsma et al. 2003; Shuker and West 2004). We have found that cues concerning whether hosts are already parasitized are much more important than social cues, such as the presence of other foundresses or the relatedness between individuals. Second, the pattern of social interactions in natural conditions can be much more complicated than that assumed by theory or laboratory experiments. More specifically, mating can be structured both temporally and spatially within patches, thereby leading to a higher likelihood of mating among individuals from the same host, which is in contrast to the usual assumption of random mating at the patch level (Shuker et al. 2005). Studies on sex ratio evolution have been extremely useful in illustrating such general points because of the relative ease with which the key parameters can be measured and linked to their fitness consequences.

APPENDIX

Table A1: A summary of the field collection

Study site and patch	No. parasitized hosts (total no. hosts)	No. foundresses	Total offspring (no. unassigned offspring)	Sex ratio for analysis
HV 8[a]	1 (15)	1	7 (0)	1.000
HV 13	27 (27)	5	607 (1)	.211
HV 220	8[b]	5	171 (0)	.199
HV 267[a]	16 (16)	7	476 (19)	.222
HV 288[c]	11 (25)	1	141 (2)	.086
HV 306	1 (6)	1	18 (0)	.056
HV 323	6 (8)	2	203 (0)	.094
HV 330	79 (82)	5	593 (3)	.197
HV 344	4 (43)	1	79 (0)	.063
HV 365	1 (35)	1	25 (0)	.160
Total HV	154 (262)	29	2,320 (18)	.186
Schl 11[c]	15 (25)	4	204 (5)	.317
Schl 13[c]	3 (25)	2	43 (6)	.108
Schl 16[c]	4 (25)	2	24 (3)	.333
Schl 20[c]	25 (25)	2	331 (11)	.178
Schl 21[c]	9 (25)	7	186 (5)	.558
Schl 22[c]	14 (25)	4	246 (1)	.188
Schl 23[c]	1 (25)	2	8 (1)	.125
Schl 28[c]	15 (25)	3	188 (2)	.048
Total Schl	86 (200)	20[d]	1,230 (33)	.241
Total	240 (462)	49	3,550 (59)	.205

Note: Wasps were collected at two field sites, either from natural host puparia found in nest boxes ("patches") or from baits, containing 25 laboratory host puparia, placed into patches. Not all of the host puparia that were found or baited were parasitized. For various reasons, not all of the offspring could be assigned to a foundress. The sex ratio is that of the assigned individuals within a patch (nest box). HV, sample collected from Hoge Veluwe National Park, Netherlands; Schl, sample collected from Schlüchtern, Hessen, Germany.

[a] These patches were ultimately not included in the analyses because the foundress in HV 8 was believed to be a constrained or virgin female and because assigning offspring to foundresses in HV 267 was problematic because of the foundresses being closely related.

[b] The number is not known because of a recording error, but it is known to be nine or more; therefore, nine is used when compiling the totals.

[c] Samples collected from baits.

[d] The total number of foundresses for Germany does not equal the sum total because six foundresses parasitized puparia in two different patches.

Table A2: Information regarding the four microsatellite primer sets used

Primer name	Annealing temperature (°C)	Chromosome[a]	Sequence 5′–3′	Fragment length (bp)	Dye
Nv-22	58	I	F) GCT ATA ACA CTT TTC CGC TCT CA / R) AAG ACC AGC TAG GGA AGA GGA TA	194–222	HEX
Nv-23	58	II	F) ATA CTC AAG CAA GCC ACA GCA TA / R) GCG TAC CAA TCC ACA GAA AAT AG	235–257	FAM
Nv-41	52	V	F) GTC AGA CGT GGG CTT TGT C / R) TTA TGC GCC ACA CAC ACC	326–358	NED
Nv-46	58	IV	F) TTA CGT CAA GGT ATA GCT GC / R) GAA TAA GTG GCT GAA AGT TCC	235–267	FAM

[a] Chromosome designation according to Rütten et al. (2004).

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