Sex ratio strategies and the evolution of cue use

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Quantitative tests of sex allocation theory have often indicated that organism strategies deviate from model predictions. In pollinating fig wasps, *Liporrhophalum tentacularis*, whole fig (brood) sex ratios are generally more female-biased than predicted by local mate competition (LMC) theory where females (foundresses) use density as a cue to assess potential LMC. We use microsatellite markers to investigate foundress sex ratios in *L. tentacularis* and show that they actually use their clutch size as a cue, with strategies closely approximating the predictions of a new model we develop of these conditions. We then provide evidence that the use of clutch size as a cue is common among species experiencing LMC, and given the other predictions of our model argue that this is because their ecologies mean it provides sufficiently accurate information about potential LMC that the use of other more costly cues has not evolved. We further argue that the use of these more costly cues by other species is due to the effect that ecological differences have on cue accuracy. This implies that deviations from earlier theoretical predictions often indicate that the cues used to assess environmental conditions differ from those assumed by models, rather than limits on the ability of natural selection to produce ‘perfect’ organisms.

Keywords: sex ratios; local mate competition; cue use; fig wasps; precision of adaptation

1. INTRODUCTION

Sex allocation is a highly productive field in evolutionary biology. Support exists in many taxa for qualitative theoretical predictions about how resource allocation to male and female reproduction should depend on environmental conditions (Charnov 1982; Hardy 2002). Also, close relationships between strategies and organism fitness permit quantitative tests (e.g. Orzack 1990; Orzack *et al.* 1991; Flanagan *et al.* 1998), more generally giving insight into the factors constraining the precision of adaptation (e.g. Herre 1987; West & Herre 1998; Herre *et al.* 2001). These studies have emphasised the need to understand how individuals assess their environment: deviations from predictions may often indicate that the cues used differ from those assumed by models, rather than limits on the ability of natural selection to produce ‘perfect’ organisms (West *et al.* 2000, 2002; West & Sheldon 2002).

Sex ratio strategies (proportion of males produced) in structured mating populations offer a chance to study how organisms assess their environment. A female-biased sex ratio is favoured because sibling males compete for mates (local mate competition, or LMC; Hamilton 1967). Potential LMC decreases as the number of females (foundresses) contributing offspring increases, so assuming they contribute equally to the brood their sex ratios should become less biased with increasing foundress density. This prediction is supported in many species, but the quantitative fit varies (see papers in Hardy 2002;

West *et al.* in press). Deviations do occur because different cues are used from those assumed by theory: in the parasitoid wasp *Nasonia vitripennis*, foundresses respond to the presence of others and to eggs already deposited in the host (Shuker & West 2004). However, the extent to which the variation in fit between species is due to the use of different cues is unknown, mainly because of limited knowledge of the latter’s causes and consequences.

Pollinating fig wasps have also been used to test LMC theory. Foundresses enter figs (enclosed inflorescences) to oviposit. Usually one egg is deposited per flower (Jusselin *et al.* 2001), and sex is maternally determined (Cook 1993). The larvae mature and mate in the fig, and then the males die (but see Greeff *et al.* 2003). They are ideal for comparative work: ca. 800 species exist with ecologies that differ only in the foundress density distribution in figs and the relationship between density and the number of wasps that mature in a fig. We term the latter brood size. Brood sex ratios are female-biased and generally become less so with increasing density, but are often more biased than predicted by theory (Frank 1985; Herre 1985; Werren 1987) that incorporates the differences in relatedness to male and female offspring that exist in inbred haplodiploids and assumes that density is used as a cue (reviewed in Herre *et al.* 1997, 2001; see also Kinoshita *et al.* 1998; Moore *et al.* 2002). In some cases this is due to data from cryptic species being combined and hence foundress densities being over-estimated (Molbo *et al.* 2003, 2004), but in others data come from single species (e.g. Moore *et al.* 2002). A possible reason in these latter species

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is that foundresses violate theory assumptions by contributing unequally to broods (e.g. Kathuria *et al.* 1999; Molbo *et al.* 2004; Zavodna 2004). When this assumption is relaxed, they are also predicted to produce more female-biased sex ratios as relative contribution and thus potential LMC increases, leading to extra brood sex ratio bias (Frank 1985; Stubblefield & Seger 1990). Average reproduction often decreases with density due to non-linear increases in brood size (e.g. Herre 1989), so relative contribution could be estimated by the number of eggs deposited. As yet, though, only Kinoshita *et al.* (2002) have shown in *Blastophaga nipponica* that foundress sex ratios both increase with density and decrease with increasing offspring number. We term the latter, which will reflect egg deposition, clutch size.

In this study, we investigate foundress sex ratio strategies in the pollinating fig wasp *Liporrhopalum tentacularis* Grandi. Zavodna (2004) foundress clutch sizes in the species are often unequal in the field. Also, consistent with the use of density and relative brood contribution or clutch size as cues to assess potential LMC, Moore *et al.* (2002) found that brood sex ratios become less female-biased with increasing density but are more biased than predicted by LMC theory when only density is used as a cue. We use microsatellite markers to quantify clutch sizes and sex ratios when two foundresses enter figs at 0.5–1.5 h intervals, and compare them to single foundress broods from Moore *et al.* (2002). We show that clutch sizes tend to be unequal within two-foundress broods, and that sex ratios do not increase with density but are related in a negative logarithmic way to clutch size. We then develop a model in which only clutch size is used as a cue to assess potential LMC, and show that our observations closely approximate its predictions when appropriately parameterised. We also report the other predictions of our model, and argue that they offer an explanation as to why cue use differs between species.

2. METHODS

(a) *Experimental organisms*

We used the University of Leeds glasshouse population of *L. tentacularis* and its host *Ficus montana* Blume for the experiments. Organisms originate from the Centre for International Forestry Research plantation, Bogor, West Java, Indonesia (06°34' S, 106°45' E) and from Rakata, Krakatau Islands, Indonesia (06°00' S, 105°27' E), and have been in culture since 1996 (Moore 2001). Around six wasp generations are produced each year, totalling more than 40 since population establishment.

(b) *Micro-satellite genotyping*

We used three micro-satellite loci (LT3-08, LT4-19 and LT4-21B) for genotyping and identifying wasp offspring (see Zavodna *et al.* 2002 for primer sequences). We extracted genomic DNA from wasps with a Puregene DNA extraction kit (Gentra, Minneapolis, USA). We carried out the amplification reaction in 20 µl volume containing 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl₂, 100 µM of each dNTP, 200 nM of each primer, 1 ng template DNA and 0.5 unit Taq DNA polymerase in storage buffer A (Promega, Southampton, UK). We used the same PCR protocols as Zavodna *et al.* (2002), except for using a MWG AG Biotech thermocycler and increasing

the amplification cycle numbers in the LT60/27 protocol to 35. We analysed fluorescently labelled PCR products on an ABI 373 (updated software ABI 377) sequencer and scored them using GENOTYPER software (Applied Biosystems, California, USA).

(c) *Introduction experiments*

To aid identification of foundresses' offspring, we first established three new sub-populations of trees with figs oviposited in by individuals with distinguishable microsatellite genotypes (tested by genotyping others from their natal fig). We maintained these for three to four generations to increase homozygosity. After this, we located figs on trees in the main population before they were receptive to oviposition and bagged them to prevent foundress entries. When these figs were receptive, we located other figs just before wasp release on trees in the three sub-populations and placed them in mesh-lidded pots. Following wasp release the next day, we allowed single foundresses from the pots to enter each bagged fig. Then, 0.5–1.5 h later, we allowed second foundresses from different sub-populations to enter each fig. We then re-bagged the figs to prevent further entries and left them until just before wasp release, when we dissected them and stored the wasps in 96% ethanol for genotyping. We carried out 25 experiments from January to March 2003, but only eight broods matured and were genotyped. Similar methods were used at the same time of year by Moore *et al.* (2002) to quantify single foundress sex ratios.

(d) *Data analysis*

We examined whether contributions differed in two-foundress broods by using *G*-tests to compare observed clutch sizes with those expected given observed brood size if contributions were equal, then summing test statistics (see Sokal & Rohlf 1995, p. 716). Because of possible between-brood differences, we examined the factors influencing foundress sex ratios with a generalised linear mixed model (GLMM) with binomial errors (library glmmPQL from Venables & Ripley (2002) imported into SPlus v.6 Professional Edition (2001, Insightful Corp, Seattle, WA)). We used the number of males as the response variable and clutch size as the binomial denominator, and fitted a model to the data with foundress density as a fixed factor, brood as a random factor, log clutch size (which explained more of the deviance than the untransformed variable) as a covariate and the interaction term between density and log clutch size. Hypothesis testing was carried out with *F*-tests.

3. RESULTS

Clutch sizes varied widely at both foundress densities: 16–117 wasps in single-foundress broods ($n=34$) and 0–43 in two-foundress broods ($n=15$: in one brood only one foundress produced offspring). Clutches were larger in single-foundress broods (ANOVA on square root-transformed data, $F_{1,47}=33.75$, $p<0.001$: single-foundress back-transformed mean=52.19, 95% CI=43.19–62.03; two-foundress mean=16.01, 95% CI=9.71–23.88). Contributions to two-foundress broods were unequal in four out of seven broods (we did not test the brood where only one foundress contributed: $G_{\text{summed } 7}=62.27$, $p<0.001$).

Sex ratios also varied widely at each foundress density: 0.01–0.37 in single-foundress broods, and 0.07–1 in

Figure 1. The relationship between *L. tentacularis* clutch size and sex ratio. Squares represent clutches in single-foundress broods; diamonds, clutches in two-foundress broods. The thick line is the log regression slope of the combined data. The thin line represents the predictions of our sex ratio model when appropriately parameterised.

two-foundress broods. They were less female-biased in two-foundress broods (GLMM: $F_{1,40}=19.58$, $p<0.001$; single-foundress back-transformed mean=0.08, 95% CI=0.07–0.09; two-foundress mean=0.21, 95% CI=0.19–0.24). However, when log clutch size was fitted as a covariate we found a negative relationship with sex ratio that explained 62% of the deviance in the data ($F_{1,6}=90.04$, $P<0.001$), but no difference between foundress densities ($F_{1,40}=0.45$, n.s.; regression slope differences: $F_{1,5}=2.87$, n.s.; figure 1). This indicates that foundresses only adjust their sex ratio according to clutch size.

4. THE MODEL

(a) Formulation

These results indicate that *L. tentacularis* foundresses contribute unequally to two-foundress broods, and adjust their sex ratio according to clutch size but not foundress density. Kjellberg *et al.* (in press) have previously argued that this strategy is used by pollinating wasps, and that sex ratios are negatively related to clutch size because as clutch size (and male number) increases foundresses produce proportionately fewer ‘insurance’ males to protect against larval mortality causing females to go unmated. Due to wasp ecology it is impossible to measure mortality, so we could not quantitatively test this hypothesis. However, it should also be noted that in these conditions clutch size supplies information about likely foundress density and relative brood contribution, and thus could be used to assess potential LMC. Optimal sex ratios under these assumptions were studied by Stubblefield & Seger (1990), but in their model (IIa) brood contribution is relative to the population mean, and it is impossible to generate quantitative predictions (the full formulation is not given). Therefore, we further investigated this hypothesis by developing a model in which clutch size is explicitly used as a cue. Also contrary to Stubblefield and Seger’s model (in which sex ratio is a continuous variable), we constrained foundresses to produce integer numbers of males and females: the often small clutch sizes in *L. tentacularis* may prevent production of optima predicted by continuous models (e.g. Greeff 1997). We specified d_i and s_i as the numbers of daughters and sons produced by a foundress in a clutch of size i , then wrote her fitness, w_i , as the number of daughters plus the number of sons

multiplied by the average number of mates they obtain, weighted by their relatednesses and reproductive values:

$$w_i = d_i G_d v_f + M s_i G_s v_m, \quad (4.1)$$

where G_d and G_s are respectively the relatedness of a daughter and a son to their mother, and v_f and v_m their reproductive values. M is the average number of mates a son obtains. It is a summation across each brood type (in terms of foundress density, brood size and pattern of foundress brood contributions) that, given i , is encountered weighted by its probability of being encountered relative to other types. Three parameters interact to determine relative encounter probability: (i) the foundress density distribution, (ii) the foundress density–brood size relationship and (iii) the probability of different foundress contribution patterns occurring. Zavodna’s (2004) *L. tentacularis* data suggest that different contribution patterns are equally likely to occur (see Molbo *et al.* 2004 for different findings in other pollinating wasps). Given this, when maximum foundress density is three (extensions are possible by using computer subroutines to cycle through brood types) M can be written as:

$$M = \frac{f_1 b_{1,i}}{N_i} \times \frac{d_i}{s_i} + \sum_{x=1}^c 2 \frac{f_2 \frac{b_{2,i+x}}{P_{2,i+x}}}{N_i} \times \frac{d_i + d_x}{s_i + s_x} + \sum_{x=2}^{c \times 2} \sum_{y=1}^{x \text{ div } 2} 3 \frac{f_3 \frac{b_{3,i+y+(x-y)}}{P_{3,i+y+(x-y)}}}{N_i} \times \frac{d_i + d_y + d_{x-y}}{s_i + s_y + s_{x-y}}, \quad (4.2)$$

where $x \text{ div } 2$ is the integer of $x/2$, and N_i the sum of the occurrence probabilities of all possible brood types given i , is

$$N_i = f_1 b_{1,i} + \sum_{x=1}^c 2 f_2 \frac{b_{2,i+x}}{P_{2,i+x}} + \sum_{x=2}^{c \times 2} \sum_{y=1}^{x \text{ div } 2} 3 f_3 \frac{b_{3,i+y+(x-y)}}{P_{3,i+y+(x-y)}}, \quad (4.3)$$

and f_n is the probability of a n foundress brood, $b_{n,z}$ the probability that a n foundress brood is of size z , and c is maximum clutch size. $P_{n,z}$, the number of clutch size patterns, given c , in an n -foundress brood of size z , was calculated by computer subroutine. Each line in the equation is a summation of the weighted number of mates in broods at a single foundress density, with the first term the brood type encounter probability and the second the number of mates.

In diploids and outbreeding haplodiploids, G_d and G_s are equal (Bulmer 1994) and can be removed from equation (4.1). In haplodiploids $v_f = 2v_m$ (Price 1970) and G_s equals 1. However, inbreeding increases G_d above its outbreeding value of 0.5 (Hamilton 1972). G_d is therefore calculated as $1/(2-a)$, where a is the probability of sib-mating (Suzuki & Iwasa 1980). To calculate a , we sum the probabilities that each type of daughter has of mating with a brother. T , the number of female offspring in broods normalised to one brood, is given by

$$T = f_1 \sum_{x=1}^c b_{1,x} d_x + f_2 \sum_{x=1}^c \sum_{y=1}^c \frac{b_{2,x+y}}{P_{2,x+y}} d_x + d_y + f_3 \sum_{x=1}^c \sum_{y=1}^c \sum_{z=1}^c \frac{b_{3,x+y+z}}{P_{3,x+y+z}} d_x + d_y + d_z, \quad (4.4)$$

Table 1. Foundress density-brood size relationships (which determine how average clutch size decreases with increasing density) used to investigate how parameter changes affect model predictions.
(Distributions at each density were calculated as a normal distribution of given mean $\pm$ s.d. wasps.)

clutch size decreases with increasing density are	foundress density		
	1	2	3
proportional	50.00 $\pm$ 15.00	50.00 $\pm$ 15.00	50.00 $\pm$ 15.00
gradual	50.00 $\pm$ 15.00	75.00 $\pm$ 15.00	100.00 $\pm$ 15.00
sudden	50.00 $\pm$ 15.00	100.00 $\pm$ 15.00	100.00 $\pm$ 15.00

and the probability that they are sib-mated is

$$a = \sum_{x=1}^c \frac{f_1 b_{1,x} d_x}{T} + \sum_{x=1}^c \sum_{y=1}^c 2 \frac{f_2 (b_{2,x+y} / P_{2,x+y}) d_x}{T} \frac{s_x}{s_x + s_y} \\ + \sum_{x=1}^c \sum_{y=2}^{c-1} \sum_{z=1}^{y-1} 3 \frac{f_3 (b_{3,x+z+(y-z)} / P_{3,x+z+(y-z)}) d_x}{T} \\ \times \frac{s_x}{s_x + s_z + s_{y-z}}. \quad (4.5)$$

Equations 4.4 and 4.5 take the same form as before: each line is a summation across brood types at a single foundress density. Using these equations, for given f_n and $b_{n,z}$ the optimal sex ratio for each clutch size can be found by dynamic optimisation. A starting population strategy is specified, and the values of d_i and s_i maximising w_i iteratively found. This is then repeated, with the optimal strategy becoming the new population strategy, until the two equilibrate.

(b) Comparison of model predictions with *L. tentacularis* sex ratios

Next, we compared the predictions of the model with our data. To parameterise the model, we quantified foundress densities by collecting *F. montana* figs and allowing wasps to emerge as before, then genotyping entire broods. Mixed paternity clutches occur rarely, if at all, in the species (Zavodna *et al.* 2005), so this does not over-estimate densities due to multiple mating. Under-estimation is possible if broods are founded by siblings, but unlikely due to high local tree densities and the large number of figs releasing wasps at any one time (Moore 2001). We collected 17 figs from the main glasshouse in Leeds in May 2001, and 16 figs from around Bogor, West Java, Indonesia in May 2002 and March 2003. Densities did not differ between glasshouse and field (GLM with Poisson errors: $\chi^2_1 = 0.13$, n.s.; glasshouse mean $\pm$ s.e. = 1.27 ± 0.45 , range = 1–3; field mean $\pm$ s.e. = 1.40 ± 0.28 , range = 1–3), so we pooled the collections. Thus, 57.6% of broods were single-foundress, 36.4% two-foundress, and 6% three-foundress. Also, we quantified the foundress density-brood size relationship in the species using Moore *et al.*'s (2002) data. Brood sizes did not increase with density and when pooled were normally distributed with a mean $\pm$ s.d. of 52.20 ± 24.74 wasps, so for each density we computed a normal distribution of these parameters (the dataset was too small to be used directly). We set c to the largest brood found by Moore *et al.* (2002): 117 wasps. Additionally, in our model the optimal strategy when clutches always occur in isolation is to produce only females, which indicates that just enough males should be

produced to mate all the females. Nefdt (1989) found that *Ceratosolen capensis* males can mate up to 20 females, so we therefore constrained foundresses to produce at least one male for this number of females.

In qualitative agreement with the data, our model predicts a negative logarithmic relationship between clutch size and sex ratio (figure 1). This is because clutch size supplies information about likely brood type and potential LMC: small clutches mostly occur in high-density broods and/or as small brood contributions, implying low LMC, and large clutches in low-density broods and/or as large contributions, implying high LMC. Sex ratios and predictions given observed clutch sizes were also quantitatively similar (paired t -test on arc-sine square root-transformed data: $t_{48} = -0.105$, n.s.).

(c) How parameter changes affect model predictions

Finally, we investigated how parameter changes affect model predictions. In all runs c equalled 100 wasps. The Poisson parameter λ in a zero truncated Poisson distribution used to calculate the foundress density distribution was set to one, three or five (average density increases with λ). Also, we constructed three foundress density-brood size relationships (computed as normal distributions with the parameters in table 1) that were similar to those found in different pollinating wasp species (respectively, Herre 1989; Kinoshita *et al.* 2002; Moore *et al.* 2002) but differed in how they caused average clutch size to decrease with increasing density. Foundresses were again constrained to produce at least one male for every twenty females. We only report predictions when both LMC and inbreeding effects are included: they are similar under only LMC, but less female-biased (unpublished results).

Under all parameter combinations negative logarithmic relationships between clutch size and sex ratio are predicted (figures 2a,b and 3). As noted in the previous section, this is because of the information clutch size supplies about likely brood type and potential LMC. Figure 2a shows predictions when the foundress density-brood size relationship causes a proportional average clutch size decrease with increasing density, and λ is increased. Predictions up to a clutch size that increases with λ remain the same. Above this and up to a clutch size that increases with λ they become less female-biased. Given the average clutch size decrease, this is a consequence of the information supplied by clutch size about likely brood type. Small clutches remain the same because they nearly always form small contributions to high-density broods. Larger clutches remain the same for longer before changing because such a decrease means that they

Figure 2. Sex ratios predicted by the model when the foundress density–brood size relationship causes: (a) a proportional average clutch size decrease with increasing density and (b) a gradual average clutch size decrease. Thick solid lines represent predictions when $\lambda = 1$; thin solid lines, predictions when $\lambda = 3$; and dotted lines, predictions when $\lambda = 5$.

continue to be more likely to occur in low-density broods (technically, as clutch size increases it becomes negatively related to the occurrence probability of high-density broods sufficiently large to contain the clutch, and therefore positively related to the increase in λ required for such broods to become common enough relative to low-density broods that greater male production is optimal). Predictions do not change above a certain value of λ because by this point clutches of the size in question already mainly occur in and are therefore optimised for conditions in high-density broods.

However, predictions differ when the density–brood size relationship causes a gradual average clutch size decrease with increasing density (figure 2b). They are less female-biased for given λ in all but the smallest clutches (which are all male), and they become less biased with increasing λ at more clutch sizes. This is because smaller average clutch size decreases mean that for given λ the probability of occurring in a large high-density brood is increased. Consequently, the information supplied by clutch size about likely brood type is less precise, and more males are produced as insurance against occurring in such broods. The probability of occurring in a high-density brood is also less size-dependent, which is why strategy changes with increasing λ occur across more clutch sizes (see also figure 3).

In addition, the model predicts that brood sex ratios at each foundress density should vary at each brood size, but overall be related to it in a negative logarithmic way (figure 4). The range of sex ratios decreases with increasing brood size and increases with increasing density. Mean brood sex ratios at each density should become less female-biased as λ increases. For given λ they should depend on the foundress density–brood size

Figure 3. Sex ratios predicted by the model when $\lambda = 3$ and the foundress density–brood size relationship causes a proportional average clutch size decrease (thick solid line), a gradual decrease (thin solid line) and a sudden decrease (dotted line) with increasing density.

Figure 4. The range of brood sex ratios predicted by the model when $\lambda = 3$ and the foundress density–brood size relationship causes a gradual average clutch size decrease with increasing density. Thick lines represent two-foundress broods; thin lines, three-foundress broods.

relationship (figure 5). In single-foundress broods they should become more biased as the amount of average clutch size decrease with increasing density increases. Provided there is a decrease they should become less biased with increasing density, with the difference increasing with the size of the decrease. We also compared model predictions with those of Werren's (1987) model where contributions are equal, density is used as a cue, and female numbers vary:

$$x^* = (n - 1)/(1 + G)n,$$

where x^* is the optimal sex ratio, n is foundress density, and G is differential foundress relatedness to daughters versus sons. $G = 2/(2 - S)$. S , the sib-mating probability, equals $1/n^*$ where n^* is average foundress density. We set λ to 3 in our model, equivalent to $n^* = 2.125$. Our model predicts more female-biased sex ratios regardless of how the foundress density–brood size relationship causes average clutch size to decrease with increasing density (figure 5).

These predictions agree with those of Stubblefield & Seger's (1990) model in terms of relationships between clutch size and sex ratio and the more female-biased brood sex ratios than when foundress density is used a cue. However, their model also predicts that sex ratios at all clutch sizes should become less biased with increasing average density. This is contrary to our prediction, and is because in their model sex ratio is a continuous variable, allowing greater precision and smaller adjustments. We

Figure 5. Mean brood sex ratios at each foundress density predicted by different models. Thin lines represent our model's predictions when λ is 3 and the foundress density–brood size relationship varies: thinner solid line represents predictions when it causes a proportional average clutch size decrease with increasing density, dashed line a gradual decrease and dotted line a sudden decrease. Thicker line represents predictions by Werren's (1987) model when n is equivalent ($=2.125$), with the single-foundress value not given because all female clutches are predicted (signifying that just enough males should be produced to mate all the females).

could not compare predictions about how the foundress density–brood size relationship affects strategies because in Stubblefield and Seger's study (which indicates a minimal effect) the foundress density distribution is simultaneously broadened in a way that will obscure any such effects. Hence, when foundresses use only their own clutch size as a cue we propose that their sex ratios should depend on both the foundress density distribution and how the density–brood size relationship causes average clutch size to decrease with increasing density.

5. DISCUSSION

Our findings indicate that *L. tentacularis* clutch sizes tend to be unequal in two-foundress broods when there is 0.5–1.5 h between entries to figs. Unequal brood contributions occur in the field in *L. tentacularis* (Zavodna 2004) and other pollinating wasp species (Kathuria *et al.* 1999; Molbo *et al.* 2003, 2004), but were previously thought due to average clutch size decreases with increasing foundress density coupled with far more sequential entries (24 h intervals: Kinoshita *et al.* 2002; see Moore *et al.* 2002 for *L. tentacularis*) or fights for oviposition sites (Moore & Greeff 2003). *L. tentacularis* foundresses do not fight (J. C. Moore, personal observation), so what causes this is unclear: perhaps the time spent negotiating the fig ostiole differs, or oviposition rates once inside. Also, our findings indicate that foundresses adjust their sex ratio according to clutch size, but not foundress density. A negative logarithmic relationship existed between clutch size and sex ratio, so the increases in brood sex ratio with density in the species (see Moore *et al.* 2002) are actually due to the accompanying average clutch size decrease, not a direct response to other foundresses. We could not measure larval mortality, so were unable to quantitatively test whether this relationship was due to the production of proportionately fewer insurance males to protect against females going unmated as clutch size (and male number) increases (see Kjellberg *et al.* in press). We did find,

though, that strategies closely approximated the predictions (when appropriately parameterised) of a new model developed in which clutch size supplies information about likely density and relative brood contribution. This supports our own hypothesis, that foundresses use clutch size as a cue to assess potential LMC when determining their sex ratio.

Do other species adjust their sex ratio only according to their own clutch size? Sex ratios in the parasitoid wasps *Trichogramma evanescens* (Waage & Lane 1984) and *Telenomus heliothidis* (Strand 1988) become less female-biased with increasing foundress density because there is an accompanying average clutch size decrease and males are mainly produced early in oviposition bouts. Furthermore, such behaviour often cannot be ruled out in other pollinating wasp species. Provided average clutch size decreases (as in Herre 1989), brood sex ratios that increase with density but are more female-biased than predicted by theory where density is used as a cue to assess potential LMC (reviewed in Herre *et al.* 1997, 2001) are predicted when both density and clutch size are used (Frank 1985; Stubblefield & Seger 1990) and when only clutch size is used (Stubblefield & Seger 1990; our model: lack of data on larval mortality again prevents quantitative predictions being made for the 'insurance males' hypothesis). Also, *Blastophaga psenes* brood sex ratios do not increase with density (Tayou 1991), which as up to six foundresses contribute before average clutch size decreases is predicted by the clutch size only models but not by the density and clutch size models. Our findings in *L. tentacularis* show that foundress, rather than brood sex ratios must be quantified if this is to be properly investigated.

However, in some species other strategies do occur. Sex ratios depend on both foundress density and clutch size in the parasitoid wasp *Telenomus remus* (van Welzen & Waage 1987) and (when ovipositing simultaneously) in the pollinating wasp *B. nipponica* (Kinoshita *et al.* 2002). Strategies in congeners *T. heliothidis* and *B. psenes* imply that these differences between species are not phylogenetically based. Instead, we believe they indicate that cue use itself is under selection, and, given our findings in *L. tentacularis*, that they reflect how the accuracy of different cues in assessing potential LMC depends on species ecology. One factor likely to affect cue accuracy is the incidence of sequential oviposition. When density is assessed by physical contact (as in the parasitoid wasp *N. vitripennis*; Shuker & West 2004), limited interaction could reduce cue accuracy to the point where its use does not evolve (in fact, in such conditions clutch size may more accurately estimate density: Frank 1985; Stubblefield & Seger 1990). Contact rates are not known for any species, but this could be why *B. psenes* does not assess density: in contrast to other species in which only 24 h intervals are recorded (Greeff & Compton 1996; Kinoshita *et al.* 2002; Moore *et al.* 2002), foundresses can enter figs at 96 h intervals (Khadari *et al.* 1995) and often re-emerge (Gibernau *et al.* 1996), so may rarely meet. Also, it could be why *B. nipponica* foundresses (who also re-emerge) use only clutch size as a cue when ovipositing 24 h after a first foundress (Kinoshita *et al.* 2002), and why *N. vitripennis* foundresses use the presence of others if the host has not been previously parasitized, but the number of already developed eggs if it has (Shuker & West 2004).

Another factor likely to affect cue accuracy is indicated by our model. Due to clutch size supplying less information about likely brood type, it predicts that optimal sex ratios should be less female-biased when there is a limited decrease in average clutch size with increasing foundress density than when there is a large decrease (the size of such decreases depends on the density–brood size relationship). Consequently, in the former conditions clutch fitness will be reduced when occurring, for example, in single-foundress broods, a reduction that if large enough could drive the evolution of density assessment. Our hypothesis assumes that it is more costly to assess density than clutch size (which seems likely given that the latter could be measured internally), but may explain why cue use differs between *L. tentacularis* and *B. nipponica*: average clutch size decreases with density in the former, but in the latter remains the same in two-foundress broods when oviposition is simultaneous (Kinoshita *et al.* 2002; see van Welzen & Waage 1987 for similar in *T. remus*). Moreover, in *B. nipponica* average clutch size is negatively related to the interval between entries to figs (Kinoshita *et al.* 2002), so the switch to using only clutch size as a cue when there is a 24 h interval could equally occur because the information supplied about likely density is more accurate in these conditions. This example shows that both the incidence of sequential oviposition and the foundress density–brood size relationship are likely to interact to determine cue use in a species, with strategies evolving to the point where any marginal fitness benefit of using other and/or more cues is outweighed by the cost (see also West & Sheldon 2002; West *et al.* 2002). Investigating this further will require the exact nature of cue use to be determined in different species, quantification of their ecologies with respect to foundress contact rates and relationships between density and average clutch size, and comparison of sex ratio strategies with theoretical predictions given different patterns of cue use.

In summary, we have provided evidence that *L. tentacularis* foundresses use their clutch size as a cue to assess potential LMC when determining their sex ratio, and that this strategy is common in species experiencing LMC. Given the other predictions of our model, we have then argued that this is because their ecologies mean clutch size provides sufficiently accurate information about potential LMC that the use of other more costly cues has not evolved, and that the use of these more costly cues by other species is due to the effect ecological differences have on cue accuracy. More generally, this has implications for the study of the factors constraining the precision of adaptation (in which pollinating fig wasps have played a central role: Herre 1987; West & Herre 1998; Herre *et al.* 2001). It suggests that deviations from earlier theoretical predictions indicate environmental conditions are assessed using different cues from those assumed by such models, rather than limits to the ability of natural selection to produce ‘perfect’ organisms.

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