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TESTING MODELS OF FACULTATIVE SEX RATIO ADJUSTMENT IN THE POLLINATING FIG WASP *PLATYSCAPA AWEKEI*

Jaco M. Greeff^{1,2} and Duncan V. K. Newman^{1,3}

¹ Department of Genetics, University of Pretoria, Pretoria 0002, South Africa

² jaco.greeff@up.ac.za

³ dnewman@tuks.co.za

Abstract

Female hymenoptera are renowned for their ability to adjust offspring sex ratio to local mate competition. When two females share a patch, they frequently produce clutches that differ in size, the female with the larger clutch optimally producing a more female biased sex ratio and vice versa. Females can base their sex allocation on their own clutch size only (“self-knowledge”) or on both females’ clutch sizes (“complete knowledge”). Few studies have genotyped offspring so that each mother’s contribution can be considered separately while none has found that both sources of information are used simultaneously. We genotyped 2489 wasps from 28 figs and assigned their maternity to one of the two foundress females. We argue that likelihood is a very convenient method to compare alternative models, while fitness calculations help to appreciate the cost of maladaptation. We find that the pollinating fig wasp *Platyscapa awekei* simultaneously uses its own as well as the other females clutch size in allocating sex. Indeed, the complete knowledge model explains the data 36 times better than the self-knowledge model. However, large clutches contained fewer males than the optimal predictions leading to a median selection coefficient of 0.01.

Key words: Sex allocation; fig wasp; clutch size; resource contest; kin selection; optimality; likelihood

Introduction

The study of sex ratios has been hailed a flagship of success of the optimality approach in evolutionary biology (West et al. 2000; West 2009). The main prediction that sex ratios should be skewed towards daughters as local mate competition between brothers increases (Hamilton 1967) is very well supported (King 1987; Herre et al. 1997; Hardy 2002). Even so, several authors have argued that while the theory seems to make qualitatively good predictions, it is quantitatively not accurate (Waage and Lane 1984; Orzack 1990, 2002; Greeff 2002; Nelson and Greeff 2009). Should we be concerned about these inaccuracies? The majority of researchers agree that the models used in the optimality approach are abstraction of reality formulated to guide our enquiries (Parker and Maynard Smith 1990). In fact, it is highly unlikely that deviations will not crop up! Many experiments reflect this view by only testing whether a proposed influential factor has the predicted qualitative effect on the trait. These tests are normally done, in Orzack's (1990) parlance, agnostically, in that the qualitative correspondence rather than the quantitative fit between predictions and data is tested.

There are pitfalls to this qualitative approach: persistent oversight of discrepancies will lead to complaisance and eventually introduce blind spots in the field (Orzack 2002). Quantitative inaccuracies are compounded by the fact that crucial assumptions are frequently not confirmed

(King 1987; Orzack 2002). In the case of sex ratios, the mating system is frequently not pinned down (Orzack 2002; Molbo et al. 2004). For instance, despite more than thirty years of sex ratio research on the wasp *Nasonia vitripennis*, the natural population structure was only investigated in two studies, one as recently as 2008 (Molbo and Parker 1996; Grillenberger et al. 2008). The qualitative approach can also lead to a myopic vision of the problem and few sex ratio studies consider alternative explanations (King 1996; Burton-Chellew et al. 2008).

Orzack and Sober (1994) have argued that the optimality principle is so pivotal a concept that it should be verified. Even so, Orzack and Sober (1994) admit that while one optimality model may be falsified, it may in principle always be possible that another would fare better. An additional problem is that statistics are geared to reject a null hypothesis rather than accept a specific alternative hypothesis. Proving that an optimality model is true is thus tricky. Orzack (1990) suggested a strong and weak test that can at least reject the optimality model. The strong test takes the correlation between predicted and observed values and Orzack (1990) argues that if this is above a high value such as 0.95, then we can "accept" optimality. The weak test calculates the regression between the observed and predicted values and if the predicted values can explain a significant part of the observed data, then the model captures at least part of reality. A potential problem with the strong test is that if the trait varies around the

optimal prediction, it will lower the correlation. We can also expect that influencing factors may be measured with inaccuracy which would introduce an error into the prediction (Parker and Maynard Smith 1990). Orzack (1990) suggests alternative ways to map behaviour onto predictions that acknowledge these inaccuracies.

A more direct approach is to measure the fitness consequences of deviations from optimality (Orzack 1990; Shuker and West 2004). If these deviations are small enough, it seems reasonable to conclude that selection has driven the trait as close to the optimal solution as is realistic. A potential problem with this approach is that, invariably, the fitness equation that was initially optimized is used to calculate the fitness. All the assumptions in the original fitness equation will thus still apply.

A potential solution to these problems may lie in the use of likelihood. Edwards (1972) has argued that the only claim we can make with some resolve is that one model/hypothesis can explain the observed data better than another. Likelihood has not been used frequently in behavioural ecology but it offers several advantages (Hilborn and Mangel 1997). First, it demands the use of alternative hypotheses. As such, it forces us to utilize one of the strengths of the optimality approach, namely to develop alternative models that take into account different sets of factors. Second, it breaks the reject-accept bind. Edwards (1972) defined support for a hypothesis relative to an alternative as the log-likelihood of the former divided by the log-likelihood of the latter. Support is thus a measurement of how much more likely

the observed data are under one hypothesis as opposed to another.

When studying adaptations, it is crucial to appreciate the ecological context of the trait (Herre et al. 2001). First, Herre (1987) showed that traits fit optimal predictions more closely in situations that are encountered more frequently and we cannot expect organisms to behave optimally in conditions they never encounter. Second, it gives us clues to the information the organism may use to facultatively adjust its strategy. By comparing data to different optimality models that consider different limitations and sources of information, it is possible to identify the information that is used. In general, and perhaps unsurprisingly, wasps seem to use more accurate information to adjust their sex ratio (Shuker and West 2004; Moore et al. 2005b).

An important extension of Hamilton's original sex ratio model was developed (Suzuki and Iwasa 1980; Werren 1980) when several workers showed that parasitoid females that oviposit after the first female produce smaller, more male biased clutches (Wylie 1966; Holmes 1972). Models show that when females know both their own and the other females' clutch sizes (complete knowledge), then the optimal sex ratio increases as the other female's clutch size increases and decreases rapidly with increases in own clutch size (Suzuki and Iwasa 1980; Stubblefield and Seger 1990; see more details in the materials and methods; Fig. 1). Stubblefield and Seger (1990) argued that it is useful to compare this complete knowledge model to the self-knowledge model where the female only has

information about her own clutch size. The self-knowledge model needs to be solved numerically and is derived in more detail in the materials and methods, but the optimal strategy corresponds to the average response of the complete knowledge model (Fig. 1).

Hymenoptera have become an established model taxon for studying sex allocation because of their life history and the fact that females can control the sex of their offspring by controlling the fertilization of eggs (King 1987; Werren 1987). Males are derived from unfertilized eggs and females from fertilized eggs. Some studies on *N. vitripennis* have considered own and other clutch size separately but have found mixed results, with either own or other clutch size significant, but not both (Shuker and West 2004; Burton-Chellew et al. 2008; Flanagan et al. 1998).

The pollinating fig wasp *Liporhopalum tentacularis* has been shown to use its own clutch size to determine sex ratios (Moore et al. 2002, 2005b; Raja et al. 2008). An earlier study claimed that females also respond to the presence of a second female (Moore et al. 2002), but this result was not repeatable in later studies (Moore et al. 2005b; Raja et al. 2008). *Liporhopalum tentacularis* lacks accurate information about oviposition by other foundresses as previous foundresses leave the fig again and additional foundresses may enter later and it determines its sex ratios by laying most male eggs early followed by mostly fertilized eggs (Raja et al. 2008). In the pollinating fig wasp *Blastophaga nipponica* second females use its own clutch size and the presence of another female to adjust their sex ratios (Kinoshita

et al. 2002). Other sex ratio studies on fig wasps have combined the clutches of the two females so that the importance of relative clutch size could not be ascertained (see Herre et al. 1997 for a review of data). However, these studies show that sex ratios increase as foundress number increases and in several species the number of males also increases, showing that the *L. tentacularis* mechanism of sex ratio adjustment is not the norm (Herre et al. 1997). Molbo et al. (2003) showed that Herre (1985) was in fact working with sets of cryptic species and that some two foundress broods could in fact contain one of each of the species. In these cases we should not expect sex ratio adjustment to an alternative species.

When oviposition sites are limited on a patch, the clutch size of the two females can be negatively correlated (Shuker et al. 2005). This will mean that self-knowledge implicitly includes knowledge about the other female's clutch size. A test of information used must thus provide control for such indirect knowledge which may be "used", but not actively perceived. Statistical analysis also needs to be heedful of collinearity of predictors.

In the case of fig wasps, the final clutch size will depend on the number of eggs the wasp carries as well as her ability to lay all the eggs. Pollinating fig wasps are pro-ovigenic (Ghara and Borges 2010) and the number of eggs a female carries correlates with her body size (Moore and Greeff 2003; Ghara and Borges 2010). Females oviposit one egg per fig ovule and can only use those ovules that have short enough styles (Nefdt and Compton 1996). In addition, females will compete for oviposition sites and will lay fewer

eggs when more females are present (Nefdt and Compton 1996; Moore and Greeff 2003). In *P. awekei*, the wasp studied here, larger females physically monopolize oviposition sites by holding smaller females in the air (Moore and Greeff 2003) and this may cause a negative correlation between the number of eggs two competing females oviposit and should increase the variance in clutch size. Variation in fig size within and between trees may be an important determinant of the number of useable ovules.

Sex ratios are commonly not precise and as the clutch size decreases the chance of a maleless clutch increases (Hardy 1992). Therefore it has been suggested that smaller clutches should be less female biased to guard against malelessness (Nagelkerke 1996). In fig wasps this relationship is very strong with 6 out of 6 species investigated by Kjellberg et al. (2005) showing a reduced sex ratio as the clutch size increased. The same trend was found in a seventh species (Kinoshita et al. 2002). This relationship can result in a decline in sex ratio as relative clutch size increases (Moore et al. 2005b). It is thus important to control for single foundress clutch size trends.

Hymenopteran males are only related to their daughters and they will benefit from skewing the sex ratio towards daughters (Hawkes 1992). In *Drosophila* it has been found that substances in the semen that influence the females have additive effects (Chapman et al. 1995). Adding these ideas together, one may expect that females who are mated more than once will produce more female biased sex ratios than those that have been mated only once.

Greeff (1996) argued that when females are mated to unrelated males, they will be less related to females than on average and should produce less female offspring. Conversely, when they are mated to brothers they will be more related to daughters and should produce more female biased sex ratios. In the fig wasp *Platyscapa awekei*, males disperse to other figs and chew holes into the figs to secure matings with females inside. These holes allow air into the figs that changes the wasps' behaviour (Nelson 2005) from which females could gain non-genetic information on relatedness to potential mates.

Here we study two foundress sex allocation behaviour of *Platyscapa awekei*, the pollinating fig wasp of the fig tree *Ficus salicifolia*. In many respects this wasp's life history is similar to that of other fig wasps and sex ratio model assumptions, but it also differs in an important way. One or a few foundresses enter the fig and oviposit their eggs. Females may fight resulting in the loser laying fewer eggs (Moore and Greeff 2003). This physical contact may allow females to assess each other's size and hence egg laying potential. When the mature wasps eclose in the fig, most of the mating occurs in the confines of the fig. *Platyscapa awekei*, however, is unusual in that some male dispersal occurs (Greeff 2002; Greeff et al. 2003; Moore et al. 2005a). Matings following dispersal will reduce the degree of local mate competition, leading to less female biased sex ratios, and may also relax selection for precise sex allocation (Nelson and Greeff 2009). After mating, females disperse and settle randomly on new receptive figs to start the cycle once again.

To appreciate the selective context of two foundress figs we determine how frequently they occur. We combine these frequency data with population genetic data to quantify the mating system. We test for a correlation between own and other clutch size and determine how much of the variation in clutch size is determined by fig, tree and foundress number. We then quantify the life time sex ratio strategies of females in the wild and use an “agnostic” approach to see which factors affect the produced sex ratios. We then calculate optimal sex ratios for complete and self-knowledge systems and use likelihood to compare how well these explain the data. We test the efficacy of Orzack’s (1990) strong and weak test and Stubblefield and Seger’s (1990) equal male number prediction.

Materials and Methods

All field work was done at the National Botanical Gardens, Pretoria, South Africa (25°44'13.08"S, 28°16'32.86"E) and all statistics and calculations were done in R2.6.1 (R Development Core Team, 2007). When generalized linear models with binomial errors for proportion data were overdispersed, quasibinomial models were fitted and an F-test was used to test for significance (Crawley 2005). In each of the two foundress figs, one of the two females was chosen randomly to serve as the focal individual. For linear models this focal female’s data were used as the response variable and the other female’s data were used as predictor variables.

VARIATION IN FOUNDRESS NUMBER

Foundress numbers were quantified monthly for a year (August 2005 to July 2006). Each monthly sample came from one tree that had a crop in the correct phase, but it could be any of the trees in the garden. Between 50 and 200 (mean = 178) figs were picked once the receptive phase had ended. Figs were split in half and the number of dead foundresses counted under a dissecting microscope.

While individual figs remain unpollinated, these figs can remain attractive to wasps for between two and three weeks (Khadari et al. 1995). But, once pollinated, figs rapidly become unattractive to other female pollinators and the ostiole becomes physically impassable. If a fig remains unpollinated for too long, it will eventually abscise. Therefore a zero-truncated Poisson distribution should fit the distribution of foundress numbers well. The Poisson distribution is determined by a single parameter, λ . For each month’s sample, we found the value of λ that maximizes the likelihood of the observed data. We did this by calculating the log-likelihood of the data for a range of closely spaced λ values (0.0001 apart).

When the rate at which wasps arrive at a tree varies over time, the negative binomial distribution should fit the observed data better (Hilborn and Mangel 1997). The negative binomial is determined by two parameters, k and r . As r increases the negative binomial distribution converges to the Poisson distribution. Similar to

above, we calculated the log-likelihood of the data for a fine grid of k and r values, with r not exceeding 200. Since the Poisson and negative binomial distributions are essentially nested, we can use the likelihood-ratio test to compare their performance.

MATING SYSTEM

A large population genetic study on *P. awekei* estimated the inbreeding coefficient, F , over 6 loci as 0.36 (Erasmus 2007). To estimate the fraction of matings by immigrant males to produce the observed inbreeding coefficient we did the following. If the proportion of sibmating in the population is S , we expect an inbreeding coefficient of $F = S/(4 - 3S)$ (Suzuki and Iwasa 1980). Conversely, the proportion of sibmating can be estimated from the inbreeding coefficient: $S = 4F/(1 + 3F)$ (Werren 1987). In our case, that means that a fraction of 0.692 of all matings should be with sibs. Assuming random mating in figs and that all females in a fig produce identical clutches, then, if all figs produce more or less the same number of female offspring, regardless of the number of foundresses, the chances of sibmating is equal to the inverse of the harmonic mean number of foundresses (Herre 1985). Alternatively, if figs with n foundresses produce n times as many female offspring as single foundress clutches, the inverse of the arithmetic mean number of foundresses gives the probability of sibmating (Greeff 2002). The truth is somewhere between these two extremes (see below; single foundress figs produced an average of 42.6 female offspring,

whereas two foundress figs produced about 1.7 times more female offspring with an average of 72.4).

WASP INTRODUCTIONS

Introductions were carried out during the summer of 2005/2006. Sampling was restricted to the summer months because Pretoria is at the edge of the distribution of *F. salicifolia* and both tree and wasp activity ebbs during the relatively cold winters. Three *F. salicifolia* trees that produced reliable crops were used for the experiment (Tree 1: 25°44'11.63"S and 28°16'31.37"E; Tree 2: 25°44'12.86"S and 28°16'48.35"E & Tree 3: 25°44'10.35"S and 28°16'33.58"E). When these trees made new figs, twigs directly exposed to the sun were selected and gauze bags tied over them. Twigs were bagged to keep out non-experimental wasps, while exposure to direct sunlight reduced fungal infections inside the figs. When the bagged figs became receptive to wasps, we collected figs in the afternoon from trees releasing *P. awekei* wasps in the Pretoria vicinity. Each releasing fig was put in a separate plastic tube with a gauze top. Female wasps that emerged that night were used the next morning for introductions.

Bags were taken off, one twig at a time, and care was taken to ensure that only experimentally introduced wasps entered figs. Twigs were assigned for single or double introductions. In the case of double introductions, single wasps were first introduced into all of the figs on a twig. An attempt was then made to introduce the second females into figs in the same

order that the first introductions were made. Sufficient time was allowed (10-20 min.) for the first female to enter, before introducing the second female. A soft paint brush was used to transfer the wasps from their releasing tubes onto a desired fig. Occasionally a wasp would not enter a fig and was replaced. Only one introduced female was used per tube of released females. Introductions were best performed in the morning and females were only used for introduction on the day of their release. When introductions were finished on a twig, the bag was securely returned.

When the non-experimental figs started showing signs of ripening, the bags were removed from the experimental twigs. Thereafter, the tree was monitored daily. Figs undergo a rapid expansion phase just before emerging wasps are released. When experimental figs started expanding, an Eppendorf tube with gauze melted over a hole cut off the apex was carefully fitted over it so as not to dislodge it. As the fig expands, it seals off against the sides of the tube. Within a matter of days the figs reach maturity and the wasps emerge (Figure S1).

During this stage, figs were checked twice daily, early in the morning and late in the afternoon. Releasing figs were picked immediately and allowed to complete releasing in plastic tubes with gauze tops. Released wasps were preserved in 96% alcohol and stored at -70°C. Wasps that failed to emerge were dissected out of their galls under a dissection microscope and added to the sample. The recorded sex ratios are thus secondary sex ratios and sex biased death during development

could lead to systematic deviations between the observed and "intended" sex ratio.

Prior to sampling, a power analysis was performed to determine a realistic sample size to collect to be confident that an effect of own and other foundress clutch size on sex ratio could be detected if foundresses do use this information when allocating sex. Computer simulation showed that a sample of 30 broods was sufficient for our purposes. In total, 93 single foundress and 119 double foundress introductions were performed. Of these, 60 single foundress broods and 88 double foundress broods were collected respectively.

MATERNITY ASSIGNMENT

Double foundress broods from each tree were randomly assigned an order. Following this order, DNA was extracted from each individual in a brood using the Chelex DNA extraction protocol developed by Estoup et al. (1996). The abdomens of female wasps that had emerged naturally were removed under a dissecting microscope. This was done as a precaution to avoid contamination from sperm in their spermathecae. Wasps were placed individually into Eppendorf tubes, frozen in liquid nitrogen, then ground using an Eppendorf pestle. 500µl of Chelex (10%; SIGMA, c7901-100G, Chelex 100 sodium form), preheated to 60°C, was added to each tube using a cut off 500µl pipette tip. Samples were placed in boiling water for 15 minutes. Thereafter, 7.5µl Proteinase K (20.3mg/ml; Fermentas, #EO0491) was added and the sample shaken down. Samples were placed in a water bath at 55°C for 1 hour and gently shaken

every 15 minutes. The extraction procedure was completed by boiling for 15 minutes.

Each individual was genotyped at 6 polymorphic microsatellite loci (Jansen van Vuuren et al. 2006) using fluorescently labelled forward primers (table S1). PCR reactions were performed in an Eppendorf Mastercycler® gradient machine in 96 well plates (table S2). Product amplification was confirmed directly on 1.5% agarose gels. PCR products were pooled for each sample and diluted to 1:10 of their original concentration. Samples were run on a Genetic Analyzer 3100 (Applied Biosystems) using GeneScan™ -500 LIZ™ Size Standard filter set (Applied Biosystems). For each sample, 1µl was added to 10µl formamide-size standard solution (1ml formamide: 10µl size standard), denatured at 94°C for 5 minutes, then placed on ice.

Fragment lengths were viewed in GeneMapper v3.5. Genotype tables were manually compiled directly from fragment profiles (bin width offset = 0.04 base pairs) for individuals within broods. Broods were examined and putative maternity was assigned to confirm foundress number. This was done by dividing males into brother groups, inferring the genotype of the mothers, then, grouping the females into their respective sister groups. Faint (relative peak height < 60) and ambiguous genotype profiles were included or excluded based on congruence with maternity suggested by corresponding loci. Individuals were only excluded from the dataset when genotype amplification failed completely at all loci or genotype profiles were ambiguous with respect to maternity.

Manually assigned maternity was confirmed using the software program COLONY (Wang 2004). Analyses were performed on the pooled genotypes of the broods from each tree. No *a priori* information was specified for the data; the interval for updating allele frequencies was set at 100; and the indicator for allelic dropout at each locus and other typing errors in the data was set at 0.05. COLONY further divided family groups into half-sibling groups when foundresses had been multiply mated. When multiple paternity was unambiguous, foundress mating status was recorded as multiply mated, otherwise as mated by one male only.

DETERMINANTS OF CLUTCH SIZE

To see if ovule availability capped the number of eggs that one and two foundresses could lay we did a two-way ANOVA with tree and female number as fixed effects and the total number of wasp offspring reared from a fig as the dependent variable. We also tested how these effects affected the average clutch size reared from figs. Post-hoc multiple comparisons were done with Tukey's honestly significant differences, coding each of the 3 tree levels x 2 female number levels as 6 treatments.

It is important to know if females from two foundress figs affect each other's clutch sizes. We fitted a linear model explaining the focal

clutch as a function of the predictor clutch, the tree identity, and their interaction.

To get an impression of how clutch size variation is determined by tree and fig, we fitted a model where tree and fig were declared as random effects with fig nested in tree. The REML method was used with parametric bootstrap (1000 resamplings) to calculate *P*-values (Faraway 2006).

NON-CLUTCH SIZE EFFECTS ON SEX RATIO

Only two-foundress broods were genotyped in order to assign maternity, so the predicted effects of multiple mating and sibmating could only be tested for two foundress figs. One female from each double foundress brood was randomly selected to be the subject representing that brood. For this subset of the data, a generalized linear model with binomial errors was fitted (Wilson and Hardy 2002; Crawley 2005). We fitted the model: sex ratio ~ tree + own clutch size + other clutch size + multiply mated + daughter heterozygosity, with tree and multiply mated as factors.

CLUTCH SIZE EFFECTS ON SEX RATIOS

Here we considered the single and two foundress broods simultaneously. For the two foundress broods the same randomly drawn focal mothers were used as above. For single foundresses, the brood size of the other female was set to zero. We fitted a generalized linear model with binomial errors to the data: sex ratio ~ tree + own clutch size + other clutch size + foundress number + all the

two-way interactions, except for foundress number and other clutch size as all the “other clutch sizes” was equal to 0 for single foundress females. Non-significant terms were dropped sequentially from this model until the minimum adequate model was obtained (Crawley 2005).

MODEL PREDICTIONS: COMPLETE-KNOWLEDGE

Suzuki and Iwasa (1980) generalised Hamilton’s (1967) model for a wide variety of situations, and by setting *p* in their model’s equation (6) equal to 1, we obtain the ESS sex ratios for two females when they lay their eggs simultaneously, but when their clutches can differ in size as:

$$x_i = \frac{1}{4} \cdot \frac{1+F}{1+2F} \cdot \frac{N_i + N_j}{2N_i} \quad (1)$$

where x_i is the sex ratio (proportion of sons) of the *i*th female when she produces a clutch of size N_i and the other female produces a clutch of size N_j , and the inbreeding coefficient in the population is *F*. The predicted sex ratio is the product of the normal biparental sex ratio of $\frac{1}{4}$ calculated by Hamilton (1967) for two females, a fraction that corrects for the increased relatedness of daughters to mothers due to inbreeding (Herre 1985), and a fraction that corrects for the females’ relative clutch sizes. As we will be testing how females adjust their sex ratio in response to both their own and the other female’s clutch sizes, it is essential to

write the last fraction in terms of both brood sizes rather than just their ratio, as is often done.

The optimal predictions are given in Fig. 1. As the other female's clutch size increases, a higher sex ratio is produced and the sex ratio falls rapidly with increases in own clutch size. We can see that if we multiply both sides of equation (1) by N_i then the complete knowledge theory predicts that the two females sharing an oviposition site should have the same number of sons ($= x_i N_i = x_j N_j = \frac{1+F}{4} \frac{N_i + N_j}{2}$; as was found by Yamaguchi (1985) and Stubblefield and Seger (1990). Another prediction that may be helpful in testing the fit between data and this model is that the last term in equation (1) can be written as $(1 + N_j/N_i)/2$ and this means that when we plot sex

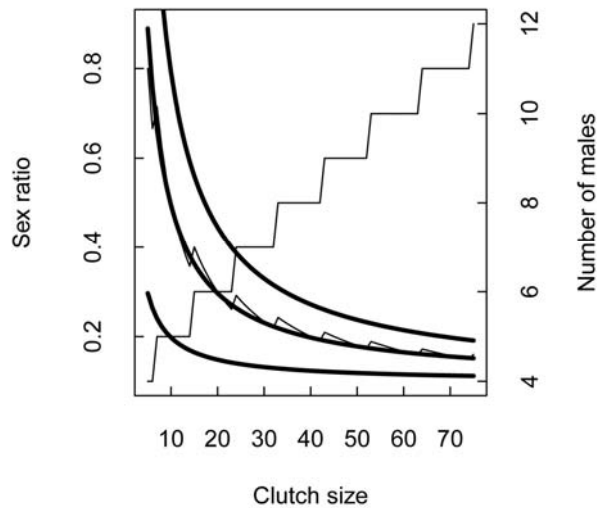


Fig. 1. The model predictions for optimal sex ratios for the complete knowledge (heavy lines, from top to bottom for $N_j = 70, 40$ and 10) and self-knowledge (saw-toothed regular line) models and the number of males for the self-knowledge model (step function increasing with increasing clutch size).

ratios as a function of (other clutch size)/(own clutch size), then it should form a straight line with an intercept and slope that are equal to each other and equal to $(1 + F)/(8(1 + 2F))$ – the straight-line prediction.

MODEL PREDICTIONS: SELF-KNOWLEDGE ONLY

To obtain optimality predictions for when females have only self-knowledge, we follow the approach of Stubblefield and Seger (1990) where the fitness is optimised over the summation of the other females' clutch sizes, each time weighted by their frequency of occurrence. The fitness of a female with clutch size i and sex ratio x_i is calculated as:

$$w_{x,i} = i(1 - x_i) \frac{1 + 3F}{2} + \frac{1 + F}{2} i x_i \sum_j \left[P_j \frac{i(1 - x_i) + j(1 - x_j)}{i x_i + j x_j} \right] \quad (2),$$

with the first term denoting fitness via daughters and the second, fitness via sons; the fractions containing F gives the relative genetic value of daughters and sons (Suzuki and Iwasa 1980; Stubblefield and Seger 1990), and the summation gives the expected mating success per male over all j patches, each with a frequency of P_j .

To obtain the optimal number of males, we calculated P_j from the distribution of 56 two foundress clutch sizes observed in this study. We set all values of $x_i = 0.25$. Then, until the optimal sex ratios stabilized, we repeatedly found the optimal sex ratio for each clutch size sequentially. The predicted sex ratio is given in Fig. 1. It is very

similar to the prediction given by the complete knowledge model for the average clutch size of around 40. The predicted number of males is also shown in Fig. 1 and it increases in a stepwise fashion with clutch size.

OPTIMALITY TESTS

We compared the log-likelihoods of observing the sex ratios under the complete and self-knowledge models. We assumed that sex ratios are binomially distributed. A likelihood ratio test can be used to obtain a P -value for this comparison. For the complete knowledge model, we obtained the optimal prediction for each clutch by inserting own clutch size, other clutch size and $F = 0.36$ in equation (1). For self-knowledge we used own clutch size to obtain the predicted sex ratios from the numerically obtained predictions plotted in Fig. 1. In both cases the log-likelihood of the data given the optimal prediction and a binomially distributed sex ratio was calculated. To see how sensitive the likelihood of the complete knowledge model is to our estimate of F , we also looked at the likelihood of a range of F values.

To determine if females made consistent errors in their sex allocation, we looked at the deviation between the observed number of males and that expected under the complete knowledge model. The model predicts a sex ratio, but the sex ratios themselves are at least binomially distributed and are not a continuous function. Therefore we compared the expectation of number of sons given the predicted sex ratio, clutch size and assuming a binomial distribution. This was calculated as the

sum of the product of the frequency of each number of sons and the number of sons.

Following Orzack et al. (1991), we used his “strong test” of optimality by calculating the correlation between the observed and predicted sex ratios. To test if this test prediction is robust in the face of binomial sex ratio variance we generated a thousand complete knowledge data sets with binomial sex ratio variation, each time calculating the correlation between the observed and predicted sex ratios. Orzack et al. (1991) also suggested a “weak test” by using the predicted values as an explanatory variable for the observed sex ratio in a generalized linear model.

We tested two specific predictions based on equation (1). First, the predicted correlation of one, between the number of male offspring between females sharing a fig was tested. Again we tested how robust this prediction would be in the face of binomial sex ratio variance by creating a thousand binomial data sets with these clutch size variants. Two, we tested the straight-line prediction by comparing the likelihood of a line with an intercept and gradient equal to $(1 + 0.36)/(8(1 + 2 \times 0.36)) = 0.0988$, to the maximum likelihood value assuming binomially distributed sex ratios.

FITNESS CONSEQUENCES

To quantify the fitness cost of deviations (binomial and unexplained), we used equation (2; except that we only summed over the current fig) to calculate the fitness of the observed strategy and the fittest strategy given the clutch composition of the other

female. This should not be confused with comparisons of the observed number of sons to the expected predictions assuming that both females produced the optimal sex ratio. This was explored under the section, “Optimality Tests”. To take into account that sex ratios are not continuous, we simply exchanged male eggs for female eggs and *vice versa* until the highest fitness was calculated. The fitness obtained by the observed strategy was then expressed as a percentage of the highest fitness. We calculated the difference between the observed number of males and the number of males of the strategy that results in the highest fitness. We used linear models to see if the difference in male number and the fitness effect of such differences are influenced by the female's own clutch size. The difference in the observed and optimal number of males was tested with a paired Wilcoxon signed rank test.

Results

VARIATION IN FOUNDRRESS NUMBER

Averaged over all the crops, 58% of figs contained a single foundress and 22% contained two foundresses. If one takes into account that two foundress figs each contain two females then the average female experiences a two foundress environment 75% (= 44/58) as frequently as a one foundress environment. One and two foundress environments are thus common and selection should have had ample opportunity to optimize

these behaviours. There was no pattern from the data that suggests that wasps could deduce foundress number from the time of the year (Fig. 2). Figs tended to either have very low arrival rates, with $\lambda < 0.75$, or with higher rates with $\lambda > 1.5$. The three crops with the highest λ were significantly better explained by a negative binomial distribution, indicating over-dispersion.

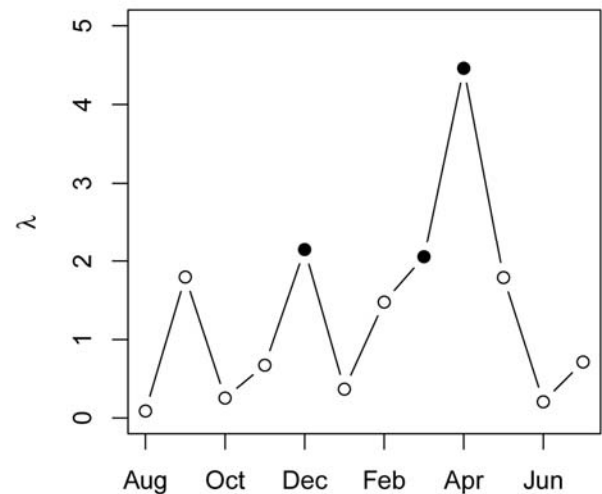


Fig. 2. The maximum likelihood estimate of λ over 12 months. Filled circles are the three cases where a negative binomial distribution explained the data significantly better than a Poisson distribution.

MATING SYSTEM

The mean of the inverse of the harmonic and arithmetic means over the twelve months are respectively 0.743 and 0.627. This means that the fraction of matings that are between sibs should fall between these two values. Our estimate from F suggested a sibmating rate of 0.694, which falls neatly between the limits of the harmonic and arithmetic approaches. Unlike our former claims (Jansen van Vuuren et al. 2006), this would

suggest that a maximum of 5% (= 74.3 - 69.4) of matings, but probably far fewer, are with immigrant males. From here on we will assume that the error introduced by this inaccuracy is negligible.

MATERNITY ASSIGNMENT

We genotyped 2489 wasps from 28 figs. Eleven female and 2 male wasps (i.e. 0.5% of all wasps), all from different figs, could not be assigned to a mother as their PCR's failed and they were deleted from the data. One fig contained an undescribed *Phylotripes* species that is parasitic on *P. awekei*. This fig had reduced clutch sizes of 15 and 16 and was removed from the data frame. Some other figs contained *Otitella pseudoserata*, but this is a galling wasp that would not have killed any of the pollinator offspring and would not have interfered with their sex ratio behaviour. Discrepancies between manually assigned family groups and those assigned by COLONY were mostly minor. Two notable exceptions led to the exclusion from further analysis of 1 brood from Tree 2 and 1 brood from Tree 3 as the possibility of the presence of a third foundress could not be ruled out (these wasps are not included in the 2489 tally). In both cases, COLONY suggested that one putative family be split in 2 based on a disagreement at 1 locus segregating for 2 alleles within the putative family. The

brother group suggested a heterozygous mother at that locus. As the sister groups were all homozygous for one or the other allele, COLONY proposed 2 mothers homozygous for each allele that were mated by males carrying the same allele as they. The implication is that these two mothers were sisters. Allelic dropout provides a more reasonable explanation for the observation. The chances of 2 sisters entering the same fig under natural circumstances is very unlikely, however the possibility could not be excluded given the nature of experimental introductions.

DETERMINANTS OF CLUTCH SIZE

Descriptive statistics of clutch sizes and sex ratios are given in Table 1. Three samples were removed from the data frame due to unusually small clutch sizes. One, was a single foundress fig containing only 9 eggs; one contained two foundresses but one female only laid 5 eggs and the final fig was the parasitized one mentioned above.

Table 1. Descriptive statistics of individual females' clutch sizes and sex ratios. Sample sizes are given in brackets.

Trait	Tree 1	Tree 2	Tree 3
Clutch size			
Single foundress	47.3 (10)	51.5 (10)	53.9 (10)
Two foundresses	38.3 (20)	36.7 (18)	59.0 (18)
Sex ratio			
Single foundress	0.120 (10)	0.222 (10)	0.141 (10)
Two foundresses	0.193 (20)	0.240 (18)	0.181 (18)

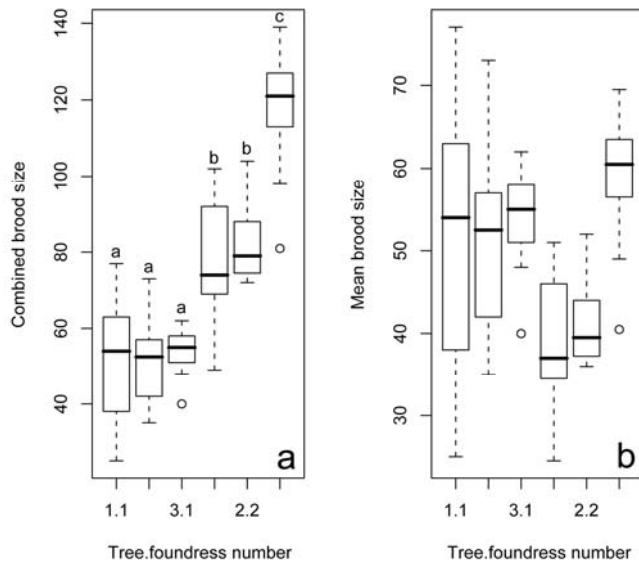


Fig. 3. The a) total and b) average clutch size per fig as a function of the tree identity, one to three, and the number of females, one or two.

Together, two females lay more eggs than single females did, but the degree to which they did so depended on the tree (Table 2, Fig. 3a). Single foundresses laid the same number of eggs regardless of the tree from which they came (Tukey HSD, $P > 0.999$). The combined clutch size of females from trees 1 and 2 was significantly larger than that for single foundresses (Tukey HSD, $P < 0.01$), but significantly less than the combined clutches from tree 3 (Tukey HSD, $P < 0.0001$).

Similarly to the total clutch size, the average clutch size was affected by both tree and female number, as well as their interaction (Table 2, Fig. 3b). Two foundress figs from tree 1 tended to have smaller average clutches than single foundress clutches from all other trees (Tukey HSD: tree 1: $P = 0.078$; tree 2: $P = 0.067$; tree 3: P

Table 2. ANOVA table for total and average clutch size. Female number refers to number of ovipositing females.

Factor	Df	SS	F	P
Total:				
Tree	3	285063	474.674	< 0.0001
Female number	1	22369	111.742	< 0.0001
Tree:Female number	2	4114	10.275	< 0.0001
Residuals	49	9809		
Average:				
Tree	3	136135	421.0512	< 0.0001
Female number	1	472	4.3804	0.0416
Tree:Female number	2	901	4.1805	0.0211
Residuals	49	5281		

Table 3. Influence of random effects on clutch size.

Groups	Variance	<i>P</i>
tree:fig	35.369	0.035
tree	118.734	< 0.0003
Residual	60.462	

= 0.018). Although the average two foundress clutches from tree 2 tended to be smaller than those from single foundress treatments, this was not significant (Tukey HSD: all *P*'s > 0.16). Conversely, two foundress clutches from tree 3 tended to be larger than those of single foundress treatments, but it was also not significant (Tukey HSD: all *P*'s > 0.62). However, the average clutch from two foundress figs in tree 3 was significantly larger than that from trees 1 and 2 (Tukey HSD: respectively: *P* < 0.001, *P* = 0.018).

Focal clutch size was determined by the tree ($F_{2,22} = 3.511$, *P* = 0.047), but there was a near significant ($F_{1,22} = 3.5927$, *P* = 0.0713) positive effect of the other clutch, i.e. in contrast to our expectation, a larger response clutch tended to be linked with a larger predictor clutch. This model had an adjusted $R^2 = 0.537$. When clutch size (as a predictor) was deleted from the model the adjusted R^2 only dropped to 0.485 (tree: $F_{2,23} = 12.756$, *P* < 0.0002). This is indicative of the two explanatory variables (tree and clutch size) being correlated (as was shown above). Although we can think of reasons why one can expect a correlated increase in the clutch sizes of females that share a fig, independent of tree effects, such as temperature or moisture content in the fig micro-environment, we conclude that it is mainly due to a tree effect

Table 4. Coefficients and significance of predictors in a GLM explaining sex ratio.

Predictor	Coefficients	<i>P</i> -value
Tree: 1	-0.648	} 0.118
2	0.467	
3	0.735	
Own clutch size	-0.046	<0.001
Other clutch size	0.022	0.035
Multiple mates (yes)	0.156	0.508
Daughter heterozygosity	-0.363	0.522

(Coefficients: tree 1 = 39.7; tree 2 = 40.6; tree 3 = 61.8). The random effects model suggests that figs explain about a 7th of the variation, tree about four 7th's of the variation and the remaining two 7th's are unexplained (Table 3).

NON-CLUTCH SIZE EFFECTS ON SEX RATIO

Two of the 28 figs contained unusually small clutches of less than 17 offspring per female. One is the fig parasitised by *Philotrypesis* mentioned above. In the other fig, one female only laid 5 eggs, whereas the other female produced a nearer to normal 45 eggs. This means that the data point can be very influential on parameter estimates. We obtained a very similar result with and without them and here report the data with the outliers. The data were over dispersed so we used the quasibinomial option with a dispersion parameter of 1.8. Accordingly, an F-test was used to test for significance (Crawley 2005). Females that could be confirmed to have mated more than once (11 of the 28 focal females) produced slightly more sons (contra our expectation) but this was not

significant (Table 4). Females with more heterozygous offspring produced fewer sons, not more as we predicted, but this trend was also not significant (Table 4).

CLUTCH SIZE EFFECTS ON SEX RATIOS

Here we excluded the two two-foundress figs that contained unusually small clutches although we obtained similar results including these figs. In addition to these 26 two foundress figs we included 10 randomly selected single foundress samples from each of the three trees. One of the single foundress figs had an unusually low offspring number of 9 and it was deleted from the data frame.

Data were over-dispersed and therefore we fitted a quasibinomial model and compared the deviance to the F-test. The MAM included tree (deviance = 83.185, $df = 2$, $P = 0.033$), other clutch size (deviance = 102.286, $df = 1$, $P = 4.7 \times 10^{-5}$) and the interaction between own clutch size and foundress number (deviance = 85.969, $df = 1$, $P < 0.004$). The predicted sex ratios are obtained as $y = e^a / (1 + e^a)$ with

$$a = \begin{bmatrix} -1.462 \\ -1.038 \\ -1.340 \end{bmatrix} - 0.007 \times (\text{own clutch})$$

for single foundress figs and

$$a = \begin{bmatrix} -1.511 \\ -1.087 \\ -1.349 \end{bmatrix} - 0.046 \times (\text{own clutch}) + 0.041 \times (\text{other clutch})$$

for two foundress figs (see Fig. 4; the array is for tree 1 to tree 3).

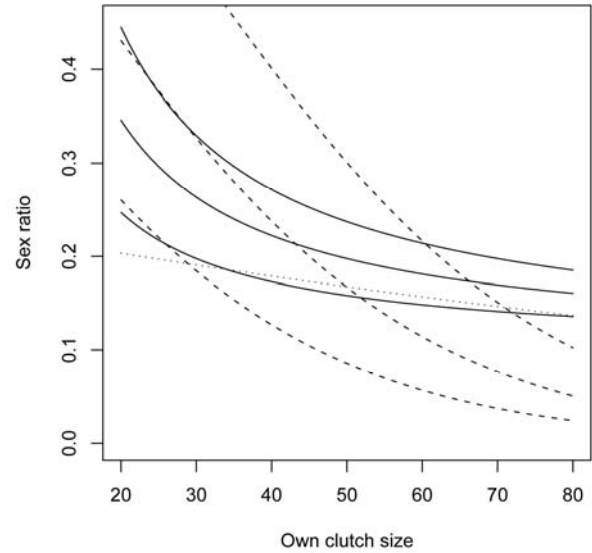


Fig. 4. Observed and predicted sex ratios as a function of own clutch size. The solid lines are the optimal sex ratio predictions for the complete knowledge model when the other clutch size is from top to bottom respectively equal to 70, 50 and 30. Dashed lines are the observed relationships given by the generalized linear model (for simplicity tree was not entered into the model depicted here). Again, from top to bottom, the other clutch sizes are respectively 70, 50 and 30. The dotted line depicts the generalized linear model for observed single foundress sex ratios (the slight negative slope is not significant).

OPTIMALITY TESTS

The log-likelihood of observing the data given the complete knowledge model is -147.2719 (= LLc), whereas the log-likelihood for the self-knowledge model is -150.8673 (= LLs). This means that the support (*sensu* Edwards 1972) for the complete knowledge model over the self-knowledge model is 3.6 and the observed data are 36 times more likely under the complete knowledge model than

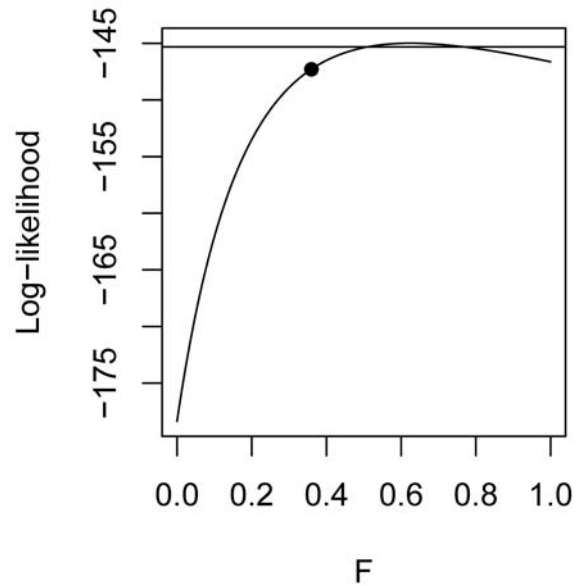


Fig. 5. Log-likelihood of data for complete knowledge model with varying values of F . The estimated value of 0.36 is indicated by the filled circle. All points above the horizontal line are significantly better than $F = 0.36$.

the self-knowledge model. The likelihood ratios test suggest that this difference is significant ($2 \times (LLc - LLs) = 7.19 > \chi_1^2 = 3.84$). The support function (Fig. 5) shows that our estimate of F ($= 0.36$), lies on the edge of a cliff, with the log-likelihood being relatively unaffected by higher values of F , but dropping sharply with lower values of F . The support is best for $F = 0.625$.

Females produced a mean of 1.3 sons less than the optimal predicted number of sons (median $= 0.81$; Paired Wilcoxon signed rank test: $V = 440$, $P = 0.024$; Fig. 6a). The deficit of males was unrelated to own clutch size (linear model: $P = 0.107$).

The correlation between the observed data and the complete knowledge prediction was 0.397

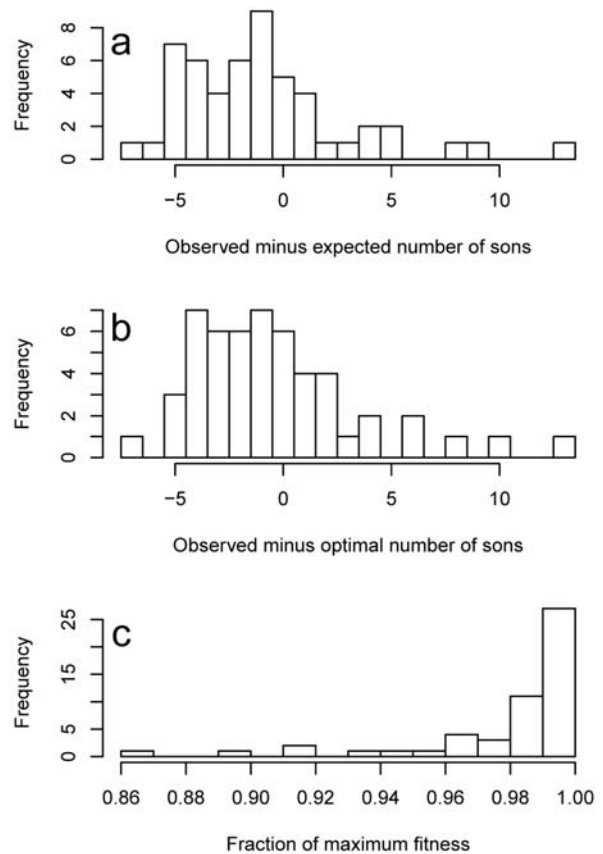


Fig. 6. a. The difference between the observed number of males and that expected from the complete knowledge model (calculated as the expectation of male number given a binomial distribution of the sex ratio). b. The difference between the observed number of males and the optimal number of males given the other female's sex ratio. c. The percentage of the maximum fitness given the other female's clutch composition.

(95% CI = 0.139 – 0.605, $P = 0.004$), whereas that between observed and self-knowledge was only 0.231 (95% CI = -0.045 – 0.474, $P = 0.099$). Neither correlation was above 0.95, thus failing the strong test. Simulation of data under the complete knowledge model indicate that with binomial variance a significant correlation can be expected

84% of the time and the average correlation is 0.387 (SD = 0.122; range -0.028 – 0.713). The complete knowledge model explained a significant amount of the variation in sex ratios of the observed data (Δ deviance = 17.13, $F_{1,50} = 8.791$, $P = 0.005$; observed sex ratio = $e^a/(1+e^a)$, with $a = -3.30 + 8.95 \times (\text{predicted sex ratio})$), whereas the self-knowledge model could not (Δ deviance = 6.03, $F_{1,50} = 2.781$, $P = 0.102$).

There was no detectable correlation between the number of sons of the females that shared figs ($\rho = -0.006$, $P = 0.976$). Simulation of data suggests that if sex ratio variance was binomial we would have expected significant correlation in only about 48% of cases. For the straight line prediction we find that the line with slope = intercept = 0.0903 explains the data best and significantly better than our expected value of

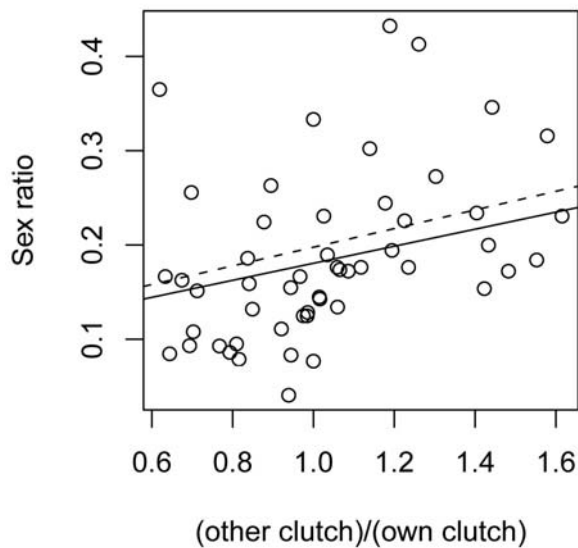


Fig. 7. The straight-line prediction: The expected (dashed) and best (solid) lines with equal intercept and slope, that explains the sex ratio as a function of other clutch divided by own clutch.

$0.0988 (2 \times (-144.9865 + 147.2719)) = 4.57 > \chi_1^2 = 3.84, P = 0.019$; Fig. 7).

FITNESS CONSEQUENCES

On average females laid 0.40 sons too few (median = 1), but this deviation was not significant (Wilcoxon signed rank test: $V = 682$, $P = 0.122$; Fig. 6b). This resulted in an average selection coefficient of 0.021 (median = 0.010; Fig. 6c). Neither of these showed a relationship with own clutch size (deviation of number of sons: Linear model of own clutch size, $P = 0.125$; percentage of maximum fitness: Linear model of own clutch size, $P = 0.72$). However, if one outlying female that produced 13 males too many is excluded, females producing larger clutches tended to

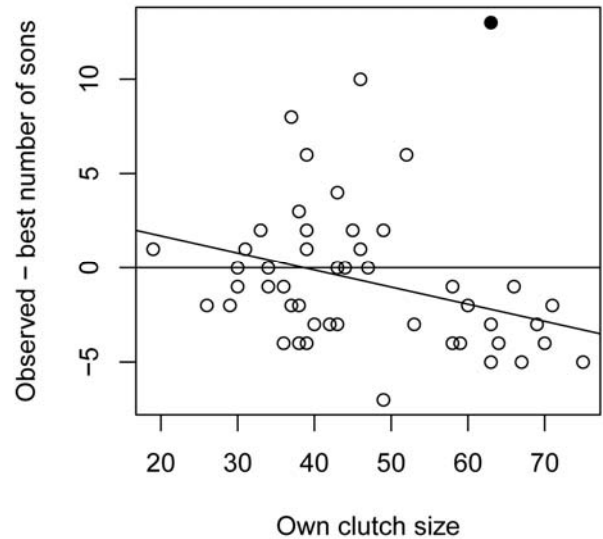


Fig. 8. The difference between the observed number of sons and the number that would give the highest fitness, given the other female’s brood against the mother’s clutch size. The diagonal line gives the significant relationship when the solid data point is disregarded.

produce too few sons (Fig. 8; Linear model of own clutch size, $P = 0.012$). If the data are divided into clutches smaller and larger than 50, the former do not produce significantly too many or few sons (Wilcoxon signed rank test: $V = 215.5$, $P = 0.974$). In contrast larger clutches contain significantly too few sons (Wilcoxon signed rank test: $V = 33$, $P = 0.041$).

For data generated from the perfect fit, but with binomial variance, the average fitness is 98.8% of the best strategy, giving a selection coefficient of 0.012. This can be compared to the 97.9% ($= 100 \cdot (1 - 0.021)$) of the observed data.

Discussion

We show for the first time that a wasp can adjust its sex ratio in response to its own and its competitors' clutch sizes. We show that the complete knowledge model explains the data 36 times better than the self-knowledge model. However, females that produce larger clutches consistently produce fewer sons than would be optimal. We must therefore conclude that although the complete knowledge model explains the data better than the self-knowledge model, it cannot account for this persistent deviation. Even so, the fitness costs are minor with a selection coefficient of 0.021.

CONTEXT OF ADAPTATION

Foundress counts throughout the year show that females encounter one and two foundress figs

frequently. As a result, selection would have had ample opportunity to fine-tune these traits and it is not unrealistic to expect them to be close to optimality (Herre et al. 2001).

Females have to adjust their sex ratios to the number of foundresses and to the relative clutch sizes. Sources of information on foundress number would thus also be beneficial. There was no seasonal trend in foundress numbers (Fig. 2) and this suggests that wasps cannot infer the density of females from climate. However, foundress numbers seemed to be either high ($\lambda > 1.5$) or low ($\lambda < 0.75$). This dichotomous pattern may be the result of receptive trees being either in close proximity to wasp-producing trees, resulting in a high arrival rate of foundresses, or vice versa. If this is the case, females may be able to predict wasp density from flight times. The three crops with the highest arrival rate also had the most variable rates (suggested by the negative binomial fit). This observation may be the result of changes in wind direction on alternate days that will have a more marked effect on wasp arrival rate if the wasp-producing tree is close by. Since *P. awekei* females do not re-emerge from figs, they have several sources of information to infer the number of co-foundresses.

We looked at females that were introduced in short succession. In a natural situation females may enter with longer time lapses (Greeff and Compton 1996). This will affect the information available to foundresses. If females enter figs sequentially the first female may not always know that a second will follow (Greeff and Compton 1996; Kathuria et al. 1999). Kinoshita et al. (2002)

showed that for *B. nipponica* the first female can adjust her strategy if the second female enters half an hour after the first, but not 4 hours later. In fig wasps where females actively leave the figs after oviposition, a second female seems to be unable to detect the former female's presence and they adjust the sex ratio solely to their own clutch size, ignoring the other eggs already laid (Moore et al. 2005b; Raja et al. 2008). Temporal aspects will also influence the information utilized. For instance, Flanagan et al. (1998) found that when *N. vitripennis* females oviposit at the same time, the focal female uses the other females' body size rather than clutch size to adjust her own sex ratio. On the other hand, when oviposition is sequential, *N. vitripennis* females appear to adjust their sex ratio to the other female's clutch size (Shuker and West 2004). Behavioural (King 1993) and genetic (Orzack and Parker 1986, 1990) data suggest that the strategy a female uses when she is the first to oviposit is independent of the strategy she uses when she is the second to oviposit on a host. Future work should quantify temporal patterns of overlap and their effects on sex ratios.

Hetero-specific females ovipositing in the same patch can lead to misinformation. *Nasonia vitripennis* is reported to reduce its clutch size and increase its sex ratio when hosts are previously parasitized by the closely related *Nasonia giraulti* (Grillenberger et al. 2009) and *Nasonia longicornis* (Ivens et al. 2009), and even more distantly related parasitoids (Wylie 1973). Similar to Wylie (1973), King and Skinner (1991b) found that pupae death leads to a reduced clutch size, but they found no difference in sex ratio. Instead sex ratio changed in

response to local cues at the site of previous oviposition (King and Skinner 1991b). These findings suggest that cross-species sex ratio adjustment cannot be a by-product of host death being the only cue used, sometimes incorrectly, to identify previous conspecific oviposition. In fig wasps pollinators frequently share the fig with other internally ovipositing species (Galil and Eisikowitch 1968; van Noort 1994; van Noort and Compton 1996; Molbo et al. 2003, 2004). Future work needs to quantify the incidence and effect of this potentially misleading biological reality.

MATING SYSTEM

Previously we have argued that as many as 15% of matings may be with immigrant males (Janesen van Vuuren et al. 2006). With a much more representative foundress count, and an inbreeding coefficient based on a much larger wasp population (Erasmus 2007), we now estimate that a maximum of 5% but possibly far fewer (1%) of the matings are with immigrants. As a result, we should not expect this non-local mating to affect our optimality predictions or our fitness calculations. Such matings should result in higher sex ratios whereas we found that females tended to lay too few males. It is thus tempting to dismiss the effects of immigrant matings altogether, but we should remember that this may be an additional source of maladaptation.

DETERMINANTS OF CLUTCH SIZES

It is crucial to establish which factors females may use to gauge own and the other female's clutch size. The data show that two females laid more eggs than single foundresses. In other words, single foundresses do not saturate the available oviposition sites. For tree three there was apparently sufficient space for two females to lay all their eggs. However, in trees one and two, each of the two foundresses laid fewer eggs than they would have if they were on their own. Despite the lack of space in these trees, and the previous report of reproductive skew (Moore and Greeff 2003), there was no negative correlation between the number of eggs co-foundresses laid. Instead, there was a positive relationship between the clutch sizes of co-foundresses, but this seems to be caused by a tree effect, rather than the co-foundress's clutch size. The lack of a correlation means that females have to assess their own and the other females' clutch size separately. In other words, if all figs allowed x eggs and the focal female laid 40 eggs, the other female would lay $x - 40$ eggs. Such a scenario would give both clutch sizes for the price of one. Furthermore, just knowing the size of the other female, potentially established through fighting (Moore and Greeff 2003), is not sufficient. The tree differences mean that females will need to take into account the size of the fig as well; in cases where space is limited, the females' relative clutch sizes will depend on their oviposition speed and possibly on interference. This result is somewhat unexpected in the light of a former study (Moore and Greeff 2003) that found that the smaller of the two females tended to lay only 70% of their eggs due to fighting. These estimates were

that single foundresses and larger females deposited an average of 67 eggs whereas smaller females deposited 45 eggs. Here we see that the average numbers of eggs that single females lay were lower, suggesting that we previously overestimated the laid eggs, or that some eggs are laid in inappropriate tissues, or that there is some developmental mortality.

NON-CLUTCH SIZE EFFECTS ON THE SEX RATIO

We found no evidence that females who mated with related males produced more female biased sex ratios. Reece et al. (2004) could not detect this relationship in *N. vitripennis* either and ascribed it to what has now been called the Crozier paradox (Aanen et al. 2008). In the case of sex ratios, the production of more female biased sex ratios when the male and female are more genetically alike leads to the loss of the genetic variation used to recognize kin. We have previously shown that the number of homozygous loci is a good reflection of sib mating history for *P. awekei* (Greeff et al. 2009). Hence, we can be confident that we should have detected such an effect if it did exist. It is possible that the presence of a tunnel is in fact not a good indicator of the presence of migrants, as we have shown that the tunnel is excavated by resident males when a small proportion of females are mated (Moore et al. 2005a). In other words, many local matings occur in an oxygen rich environment. Nelson (2005) also showed that males are very inactive under lower oxygen conditions of sealed figs. In addition, if migrant

matings are very infrequent, certainly less than 5% of time, then the wasps may not be adapted to this source of information. This will force females to use kin recognition cues rather than indirect cues to determine kinship.

We found no evidence that females that were mated more than once produced more daughters. This suggests that although males only pass genes on to daughters and would thus favour a more female biased sex ratio, they do not manage to do so, or at least that if they do so, it is not via substances in the ejaculate that work additively. Shuker et al. (2006) found that only 2% of sex ratio variation can be explained by a difference in male lines in *N. vitripennis*. These findings, together with the fact that hymenopteran mothers can commonly adjust their sex ratio facultatively, lead us to conclude that females normally win this arms race.

QUALITATIVE AGREEMENT WITH THEORY

In line with the predictions of theory (equation 1), we find that females increase their sex ratio as the other female's clutch size increases and decrease their sex ratio as their own clutch size increases in size. This is the first illustration that a wasp can respond to both of these sources of information. A number of studies, all on *N. vitripennis*, compared sex ratios to own and other clutch size and yielded conflicting results: Shuker and West (2004) found an increase in sex ratio as the other clutch size increased, but no effect of own clutch; Burton-Chellew et al. (2008) found a decrease in sex ratio as own clutch size increased

but did not test for an effect of other clutch size; Flanagan et al. (1998) found a decrease in sex ratio as own clutch size increased, but no effect of other clutch size. Part of the answer may be in biological conditions of experiments (King and Skinner 1991a; Greeff 1996; Flanagan et al. 1998), but it could also result from a correlation between own and other clutch sizes.

Most tests of these models have used Werren's (1980) formulation which gives the optimal sex ratio in terms of the relative clutch size of the two females. When Suzuki and Iwasa (1980) tested their model against the data of Holmes (1972) they also plotted sex ratio against relative clutch size. Relative clutch size has the advantage that it can be depicted on a single axis, but it has the drawback that it confounds the effects of own and other clutch size. Hence, when sex ratios decline as relative clutch size increases (Suzuki and Iwasa 1980; Werren 1980; Waage and Lane 1984; Orzack 1986; Flanagan et al. 1998; Burton-Chellew et al. 2008), it is unclear what information the female wasp is using: just her own clutch size or also the other female's. In future work it is crucial to test and report these two factors separately.

If single foundresses lay less female biased clutches as their clutch size decreases and if there is a limit on oviposition sites per host, then females will "automatically" adjust their sex ratio to own and other clutch size. This sex ratio trend is common in fig wasps (Kjellberg et al. 2005) and others: across bethylid wasp species (Hardy and Mayhew 1998), but not in Ichneumonidea (Smart and Mayhew 2009). Three of 11 bethylid species

showed a negative relationship within species (Mayhew & Godfray 1997; Hardy et al 1998). *Nasonia vitripennis* does not seem to show this relationship (King 1987; Shuker et al. 2006). Hence, to ascertain that females are actively adjusting their sex ratios, it is crucial to illustrate a significant interaction term between clutch size and number of foundresses. This means that females are behaving differently in a two foundress situation and that a fixed relationship between sex ratio and clutch size as observed in the pollinating fig wasps *L. tentacularis* (Moore et al. 2005b; Raja et al. 2008) cannot explain our observation. *Platyscapa awekei* may thus be similar to *Nasonia vitripennis* where first and second sex ratios seem to be independent traits (Orzack and Parker 1986, 1990; King 1993).

QUANTITATIVE AGREEMENT WITH THEORY

By comparing the likelihoods of the complete and self-knowledge models, we showed that the data are 36 times more likely under the former model. This simple answer underscores the strength of the likelihood approach advocated by Edwards (1972). Using the likelihood approach also forces us to compare the performance of multiple models. If anything, we hope that this paper will convince more people to use likelihood to test alternative models.

Orzack's weak tests suggest that the data can be explained by the complete knowledge model, but not by the self-knowledge model. Together with the likelihood approach above, it is thus clear that the complete knowledge model

explains the data better than the self-knowledge model.

We showed that two formerly suggested tests are uninformative in the face of binomial sex ratio variation. Yamaguchi (1985) and Stubblefield & Seger (1990) showed that co-foundresses should lay equal numbers of sons, but when we take binomial sex ratio variance into account, and possibly the low variation in combined clutch sizes, the equal-sons test may frequently not hold. Orzack (1991) suggested that model fit can be evaluated by a correlation of predicted and observed sex ratios exceeding a high value like 0.95. However, we found that when binomial sex ratio variance is allowed, perfect data had an average correlation of only 0.39 and was not even significant in 16% of simulated cases.

A new test we propose, the straight-line prediction, shows that the observed sex ratios are significantly lower than the model predictions. By comparing predicted and observed numbers we show that females indeed produce an average of 1.3 sons less than the complete knowledge model predicts. In the fitness analysis we found that it is mostly the larger clutches that have too few sons whereas the smaller ones are not different from the best strategy. *Platyscapa awekei* suffers from mild inbreeding depression (Nelson 2009) and this may explain part of this deviation. If females are unable to recognize kin but suffer from inbreeding depression, then mothers can reduce sibmating in cofoundress figs by specialising in one of the sexes. Models (unpublished data, JMG) show that the females with the larger clutch should specialise in females while the smaller one should specialise

in males. However, this effect is very small and seems unlikely to explain the discrepancy.

Fig wasps frequently produce more female biased sex ratios than expected (Frank 1985; Herre et al. 1997; Kinoshita et al. 1998). These sex ratio expectations may be too high if the level of inbreeding estimated from ecological data is too low. Ecological estimates will be too low when clutch sizes are unequal (Frank 1985; Zavodna et al. 2007) and when females enter figs, but fail to oviposit (Molbo et al. 2004). Note that this explanation is not valid here as we measured the relative clutch sizes and independently estimated the degree of inbreeding.

FITNESS CONSEQUENCES

Since *P. awekei* females lay all their eggs in a single fig, we have effectively recorded the lifetime fitness consequences of their sex allocation. This is with the exception of immigrant matings, but we have shown that these will account for a small fraction of fitness. As the sex ratio approaches the optimal ratio, the selection coefficient will become smaller. Also, the fitness of a sex ratio strategy is dependent on the sex ratio of the other female. Therefore we made the distinction between the best sex ratio, which is the sex ratio giving the highest fitness, given the actual sex ratio of the other female's strategy, and the optimal sex ratio, which is the evolutionary stable sex ratio assuming the other female also produced the ESS sex ratio. The median selection coefficient was 0.01, which is less than half the average value of 0.021. This difference stems from a few females

that laid far more sons than the optimal prediction. It is possible that these females had too few sperm to fertilise all the "intended" female eggs (West et al. 1997). Additionally, part of the deviation from the best sex ratio is caused by variance in the sex ratio trait. Assuming optimal behaviour with binomial variance we obtain a selection coefficient of 0.012. Comparing this value to the observed selection coefficients, it seems that the majority of the selection stems from sex ratio variance rather than a lack of model fit.

CONCLUSION

We have argued for a number of standard operational procedures that are required for sex ratio studies. First, correlations between own and other clutch size needs to be quantified. Second, it is important to keep own clutch and other clutch as separate predictors, rather than taking their ratio, as it allows us to see how each source of information is used. Third, we recommend that likelihood can be used to compare the efficiency of alternative models. Fourth, direct comparisons to optimal predictions and its fitness consequences can yield interesting insight into how sex ratios are non-optimal. Using this approach we show that *P. awekei* females that share a fig with another female use their own as well as the other female's clutch size to allocate sex. However, their allocation is suboptimal with females producing large clutches, producing too few sons. The growing body of sex ratio studies on fig wasps and other parasitic wasps, notably *N. vitripennis*, suggests that sex ratios can be pleasingly close to optimality

predictions, but that the predictions are nuanced by peculiarities of each species' biology.

ACKNOWLEDGEMENTS

The running costs were funded by National Research Foundation (NRF) grants 2053809 and 2072974 to JMG. DVKN was supported by a free-standing Masters scholarship from the NRF. We thank the NRF for this support and note that any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Research Foundation. We are grateful to The National Botanical Gardens of South Africa on whose premises experimental work was conducted. JMG thanks Colette St. Mary and Adnan Moussalli for respectively introducing him to *Ecological Detectives* and *Likelihood*. DVKN thanks Motoaki Kinoshita for technical assistance during sample collection. DVKN collected all the data, did molecular work, maternity assignments and initial statistics. JMG conceived the study, did modelling, statistics and likelihood calculations. Both authors wrote the paper and we benefited from the thoughtful suggestions of Stuart West, Dave Shuker, Mary-Louise Penrith and an anonymous reviewer.

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Supplementary material

Figure S1 Releasing wasps from introduction experiments were caught in traps made from Eppendorf tubes. Figs undergo a rapid expansion phase just before releasing, sealing traps over the figs. Caught wasps can be seen in the lower right trap. Traps were made by cutting off the apex of the tube and melting a fine gauze mesh over the end. Traps were loosely fitted over figs as they approached ripeness and were examined twice daily once figs started releasing.

Table S1. Primer sequences, size range and allelic diversity of the 6 microsatellite loci used to assign maternity to individual wasps collected from experimental double foundress broods.

Name	Primer sequence (5'-3')	Label colour	Size (bp)	# alleles
Pa 1	F: GTA GCG CCG TAT CAA ATT GCA A R: GGG AAG CTT GGG ATC TTT AAC GA	Green	225 - 272	20
Pa 4	F: GGG TGT TGT CGG TTT GTG AGA R: GGC AAA CAT CCA TCG GAG TGA	Yellow	192 - 233	28
Pa 7	F: CTG CCG GTC AGA GGA GGA A R: TAT GAC GTC ATC GGT TTG GCA A	Blue	235 - 345	30
Pa 8	F: GAG GAA GTC CGA TGA ATG AAC GA R: GCG AAC AGG AGA CAA AGA CAG A	Blue	191 - 217	12
Pa 21	F: GCT GTC GAG GCG AAA CAC A R: GCG CGA GGC ATT GGC AA	Green	160 - 222	39
Pa 32	F: CGG TGT TCA ATT GCC AAG TGA R: TCG TGT TCT TCG TAA TCG CGT A	Yellow	107 - 150	30

Table S2. PCR reaction conditions for the 6 microsatellite loci used to amplify and genotype the individual wasps collected from double foundress broods. Two different Taq polymerase enzymes were used: Roche, Expand High Fidelity PCR System, 1 732 650 and AmpliTaq Gold® with GeneAmp®.**Roche, Expand High Fidelity PCR System, 1 732 650**

Reagents	Quantity
Genomic DNA	0.5µl template
buffer + MgCl ₂	1x
Primers	0.3µM
dNTP's	0.16mM
Taq DNA Polymerase	0.5U

Table S2 continued

Reagents	Quantity
Reaction volume	10 μ l
Reaction steps	Conditions
Hotstart	2 min., 95°C
Cycles	[95°C, 40 sec; 60°C, 1 min.; 72°C, 2 min.] x 29
Final step	72 °C, 1sec.
Hold	4°C

AmpliTaq Gold® with GeneAmp®

Reagents	Quantity
Genomic DNA	0.5 μ l template
Buffer	1x
MgCl ₂	pool 1: 2mM; pool 2: 1.8mM; pool 3: 2mM; pool 4: 1.5mM
Primers	0.3 μ M
dNTP's	0.16mM
Taq DNA Polymerase	0.5U
Reaction volume	10 μ l
Reaction steps	Conditions
Hotstart	10 min., 95°C
Cycles	[95°C, 40 sec; pool*°C, 1 min.; 72°C, 2 min.] x 30
pool 1:	50°C
pool 2:	65°C
pool 3:	63°C
pool 4:	60°C
Hold	4°C
Reaction pools	Primer(s)
1	Pa 1
2	Pa 4
3	Pa 7, Pa 8, Pa 21
4	Pa 32