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A Study on the Sex Allocation Behaviour of the Pollinating Fig Wasp, *Platyscapa awekei*

by

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Summary

The behaviour of sex allocation has been extensively studied in hymenopterans (ants, bees and wasps) as an adaptive trait with respect to intra-specific competition within the framework of kin selection theory. Mating in these organisms often takes place in patchy populations established by the offspring of a few foundresses. Typically, there is a bias in favour of female dispersal from these patches. Theory predicts that foundresses that oviposit alone will do best to produce just enough sons to mate all of their daughters so as to maximize the number of dispersing daughters, under conditions of what is referred to as Local Mate Competition (LMC) between brothers to mate their sisters. If foundresses co-found a patch with other foundresses, they are expected to invest more resources in sons insofar as opportunity to sire offspring with the daughters of the other foundresses presents itself.

Among organisms with such a life histories are fig wasps, the insects that pollinate and lay their eggs in the flowers that grow inside young figs. There is thought to be strong selective pressure for foundresses to use information about clutch size differences in species where clutch sizes are small and low foundress numbers are frequently encountered. However, less rigorous modes of sex allocation are thought to suffice in species encountering intermediate foundress numbers. Theory thus predicts a positive relationship between the degree of structure within mating populations and the information utilized by foundresses with respect to intra-specific competition for resources and mating opportunities. This is being extensively tested across the diverse species range of fig wasps and their hosts with the larger objective in mind of contributing to a better understanding of the role of natural selection in accounting for variation observed of intra-specific behaviour.

This dissertation reports on a study of the sex allocation behaviour of the pollinating fig wasp *Platyscapa awekei*, a species characterized by low foundress numbers and clutch size differences brought about by foundress competition over oviposition sites. Offspring collected from experimentally controlled two-foundress broods were fingerprinted using microsatellite genetic markers to assign maternity and work out clutch size differences. These data are used to test what information foundresses use when allocating sex. It is reported that foundresses appear to use information of clutch size differences in two foundress broods. This observation provides evidence of advanced information utilization in fig wasps. More generally, the findings add support to

the hypothesis that natural selection can bring about subtle adaptive behaviour at the individual level, but simultaneously highlights the importance of accounting for the selective regime of the organism being studied when attempting to understand the role of natural selection in the evolution of fine scale adaptive traits.

Literature Review

Introduction

The theory of kin selection (Hamilton, 1964) provides an explanation of how altruistic behaviour can evolve whereby self-sacrificial behaviour is selected for when the cost of altruistic action to the donor is out-weighted by the benefit to the recipient multiplied by the degree of relatedness between the two parties. While the benefits of a group pulling together in the face of adversity are obvious, it is not always clear that abuse of this strength is likely to ultimately lead to its demise if it is not checked. Similarly, at an individual level, selfish behaviour might be seen to be an evolutionary dead-end. This is alluded to by Hamilton (1967) where he shows that selfish genes will increase fantastically initially, but then plunge into extinction as the host of their exploitation is exhausted. The question seems to be left begging as to what behaviour can be said to be adaptive.

The economic theory of games has shed some light on this problem by analysing the outcomes of different behavioural responses to situations of conflict arising over limited resources. While group selection has been invoked to explain altruistic behaviour in the past (Lorenz, 1963), behaviour at the group level is ultimately determined by the behaviour of the individuals of which it is comprised (Price, 1970). It can be illustrated mathematically that the behaviour that maximizes the long-term fitness of an individual will also typically be in the best interests of the group (Maynard Smith & Price, 1973). For example, it has long been a mystery why animals that are lethally armed often only engage in ritualized assessments of strength to resolve conflicts, not least of which are over mates, where it would seem that escalation of force will bring reward. By responding to force only in so far as the challenge demands, an individual is least likely to incur injury and is duly rewarded (Maynard Smith, 1976).

An extension of this theory is that natural selection should thus bring about adaptation to the environment and homogenisation of behaviour even at the individual level. Just as species are observed to fit to their environment, cases of diversity of behavioural interactions between individuals within species can be understood to be adaptive. A better understanding of the dynamics of the forces that determine the boundary of the influence of natural selection within the framework of the theory of evolution is thus a field receiving much academic attention (Orzack & Sober, 2001). With respect to interaction between individuals within species, the study of the behaviour of sex allocation has become an important model system for making and testing theories of adaptation at this level.

Sex allocation as an adaptive trait

Darwin (1871) noted an equal number of males and females in many mammalian species. The consistency of this observation led him to suspect that it be due to adaptation. He, however, could not come up with an adequate explanation of how the allocation of resources to the sexes relates to fitness (Darwin, 1874). Fisher (1930) showed that one has to look further to the reproductive success of their offspring with respect to the prospect of finding a mate. In populations where there is a shortage of one sex, a mother with an inclination to produce a higher frequency of the under-represented sex will be selected for. As a result, this sex will become more common until a point is reached where the marginal returns on investment in sons and daughters are equalised at the population level.

If the two sexes are equally costly to produce, the population sex ratio [sons/(sons+daughters)] will equilibrate at 0.5 (Shaw & Mohler, 1953). At this point, there is no difference in fitness between females producing different sex ratios. However, females producing an equal proportion of the sexes are predicted to predominate because they will always be fitter than average when the population deviates from equilibrium. Modelled under the assumption of infinite population size and random mating (panmixis), these conditions are met for many large animals. Many other organisms, however, follow very different life cycles.

Hamilton (1967) extended sex ratio theory to explain the observation of female biased sex ratios in highly inbreeding species where only one sex disperses. Many arthropods grow up in confined spaces where they will mate in so-called ‘patchy mating populations’, comprising the offspring of a few foundresses. When the young mature on a patch, they mate among themselves; the daughters disperse to repeat the cycle and the sons typically do not migrate from the patch. Under these conditions, the possibility that brothers will compete to mate with a sister becomes real. This phenomenon is referred to as Local Mate Competition (LMC).

Foundresses will potentially produce more grandchildren by trading in the resources invested in sons that are likely to lead to LMC to produce daughters that can disperse. At the same time, however, sons can sire offspring through the daughters of the other co-foundresses, while the possibility that daughters will compete for resources on future patches also needs to be considered (Taylor, 1981). In a simple model assuming equal clutch contributions and simultaneous oviposition, the optimal sex ratio for a foundress to produce thus depends on the number of co-foundresses in a patch (Hamilton, 1967). When a foundress is alone in a patch, she is theoretically expected to produce only daughters. In reality, she should produce as many sons as are required to mate all of her daughters (Hamilton, 1967). As foundress number increases, the optimal sex ratio quickly increases then gradually approaches 0.5 as the patch becomes effectively panmitic.

Since the proposal of this theory, many examples of species experiencing different levels of inbreeding have been documented, exhibiting population sex ratios showing a close fit with theory. The same principle also applies within mating patches and, remarkably, studies documenting variation in foundress number within species showed that as foundress number increases, brood sex ratio increases (Suzuki & Iwasa, 1980; Werren, 1980; Herre, 1985, 1987; Frank, 1985; Yamaguchi, 1985). Thus, while species may be seen to be generally adapted to average levels of LMC experienced, it is through the conditional response of individuals to experienced LMC intensity that we perceive the general response. Many different organisms are now being studied within this framework (Hardy, 2002) and many different sex determination mechanisms are now known that potentially allow foundresses to have control over the allocation of sex (Charnov, 1981).

Hymenopteran sex allocation

Female hymenopterans are able to determine the sex of their offspring by controlling the fertilisation of their eggs. Unfertilised eggs develop into sons and fertilised eggs into daughters (Werren, 1987). Much of the pioneering work on sex allocation was done on the parasitic wasp *Nasonia vitripennis*. Female wasps of this species lay their eggs on other fly pupa that they paralyse by stinging. The eggs mature on a host; the emerging adults mate among themselves; then, the females disperse to found new populations. Larvae are either singly or doubly parasitized and in this system clutch size differs depending on the order of oviposition.

The first foundress to arrive lays a large clutch while a second foundress is typically restricted to laying a small clutch in the remaining space that is available. Entomologists have known for a long time that foundresses that oviposit first produces a female biased clutch, while foundresses that oviposit on a parasitized hosts produce male biased clutches (Werren, 1980; King, 1993). If foundresses are able to assess the resource supporting capacity of a host, relative to their own fecundity with respect to arrival order, theory predicts that foundress that oviposit first should produce a sex ratio that is an increasing function of probability of the arrival of a second foundress and the anticipated clutch size difference should a second foundress arrive (Werren, 1980). Using the same logic, second foundresses are expected to produce a sex ratio that is determined by the expectation of the sex ratio produced by the first foundress and her relative clutch size (Werren, 1980; Suzuki & Iwasa, 1980).

These predictions fit closely with the observed behaviour in *N. vitripennis* and have been supported by numerous studies (reviewed by Werren, 1987; King, 1993; Flanagan *et al.*, 1998) that present evidence in favour of the hypothesis that natural selection is able to bring about behaviour that is locally optimal. Interpretation of the generality of these observations has, however, been questioned. Following extensive studies showing heritable variation in sex allocation behaviour observed across different laboratory strains of *N. vitripennis*, Orzack *et al.* (1991) campaigned for tests of adaptive optimality to include quantitative assessments of fit between models to data and to present evidence that natural selection could reasonably have brought about such a fit when it is observed (see also Orzack & Sober, 1994).

Studies attempting to account for observed variation around predicted responses have led to revision of simplified assumptions about the mating systems of the model organisms being studied and have identified other influences on sex allocation besides LMC (Frank, 1985; Hardy, 1994). Notably, the asymmetric value of sons and daughters (Trivers & Willard, 1972; Werren, 1984, Werren & Simbolotti, 1989) on different quality hosts and, in hymenoptera, the increased relatedness of mothers to daughters relative to sons with increasing inbreeding (Suzuki & Iwasa, 1980; Herre, 1985; Frank, 1985) have improved model fits. At the same time, these improvements have also served to re-emphasise the importance of interpreting adaptation within the context of the selective regime being studied (Herre, 1987).

These findings show the nature of adaptation of a trait is a relative concept and is typically correlated with the frequency with which the relevant environment is encountered in nature (Herre, 1987; West & Herre, 1998a). What is adaptive under one selective regime may not be selected for under another. Just as other evolutionary forces besides natural selection are important in determining the outcomes of evolution at higher taxonomic levels, so these forces are similarly at play within individuals and contribute to the diversity of behavioural responses observed between individual within species (Frank, 1986). Work on different organisms is thus interesting to see if similar kinds of adaptation have evolved under similar circumstances and how often this has happened. In this capacity, fig wasps have strong potential to address this question.

Fig wasps

Female fig wasps lay their eggs in the flowers that bloom inside young figs (Ramirez, 1969). Depending on the species of fig tree, only a certain number of foundresses enter a fig. They enter through a small hole at the apex of a fig, called the ostiole. The passage into a fig is difficult and once inside, a wasp typically cannot re-emerge and will have lost her wings. Foundresses will pollinate some of the flowers with pollen carried from the figs where they hatched (Herre, 1989). Once this task is completed, foundresses die within the fig. The larvae develop as the fig develops and hatch just before the fig becomes ripe. The young adults mate amongst themselves after which the males chew an exit hole in the wall of the fig and the females disperse to repeat the cycle. The fig quickly ripens and will likely be eaten by another animal. In the process some of the seeds will be dispersed and a new fig tree may grow. There are 750 or more different fig species and many unique pollinators (Cook & Rasplus, 2003).

Just as fig trees range in size and distribution, so foundress number and life history of the wasps also varies. Males seldom disperse (but see Greeff *et al.*, 2003) and a fig is typically receptive for only a brief period leading to the assumption of simultaneous oviposition (but see Greeff & Compton, 1996; Kinoshita *et al.*, 2002). Similar to other hymenopterans, brood sex ratio is seen to increase with an increase in foundress number (Herre, 1985, 1987; Frank, 1985). Under the assumption of simultaneous oviposition and equal clutch contributions, it has been proposed that foundresses ‘count’ the number of co-foundresses when allocating sex (Hamilton, 1979; Frank, 1985; Herre, 1985, 1987).

Unlike some other organisms, it has not been so easy to study the sex allocation behaviour of fig wasps with relation to simultaneous oviposition and clutch size differences. The reason for this is that one has to open a fig to observe oviposition behaviour and this disturbs the wasps and precludes the development of larvae. If one leaves the fig intact, one cannot know if there are clutch size differences between foundresses, or if individual sex ratios differ, without assigning maternity to broods. Toward addressing this difficulty, several indirect studies have made significant contributions. Notably, it has been shown that foundress clutch size can vary within patches and is often correlated with asynchronous oviposition (Greeff & Compton, 1996; Kathuria *et al.*, 1999; Kinoshita *et al.*, 2002).

When oviposition is asynchronous, clutch size typically decreases with arrival order due to oviposition site limitation (Nefdt, 1989). These observations have led to reassessment of the assertion that foundresses 'count' the number of co-foundresses when allocating sex. Researchers have suggested that foundresses might be producing a constant number of males and that the increase in brood sex ratio may be caused by the progressive decrease in clutch size with the order of foundresses arrival at a patch (Nefdt, 1989; Moore *et. al*, 2002). Thus, foundresses could be producing a roughly appropriate sex ratio for the LMC they experience by virtue of the clutch size they lay, rather than directly counting co-foundresses. In nature, however, it is most likely that a combination of foundress number and sequential oviposition will be experienced to give rise to clutch size differences.

Kinoshita *et al.* (2002) studied the sex ratio and clutch size difference in a pollinating fig wasp using a novel reciprocal experimental design. Foundresses were introduced singly or doubly into figs and allowed to develop. In the case of double foundress introductions, foundresses were either introduced simultaneously or sequentially at differing time intervals. By using radiation to sterilise one of the foundresses, the response to clutch size variation and oviposition order was distinguishable. Kinoshita *et al.* (2002) showed that while foundress sex ratio decreased with clutch size, foundresses from multi-foundress broods that oviposited equivalent clutches produced different sex ratios depending on the time delay between introductions. Foundresses in this species thus show sensitivity to foundress number and are not simply producing males first.

The finding suggests that fig wasps may be capable of conditionally controlling sex allocation in a manner comparable to other arthropod taxa where foundresses use information of clutch size difference in multi-foundress broods (Suzuki & Iwasa, 1980; Yamaguchi, 1985) and particularly those reported in *N. vitripennis* and other parasitic wasps (Werren, 1987; King, 1993; Flanagan, 1990). Stubblefield and Seger (1990) refer to this level of information utilisation as 'Complete Knowledge' and distinguish between random mating within patches and complete avoidance of sib-mating. It is expected that for this information to be effectively used, precise sex allocation will be greatly advantageous (Nagelkerke, 1996). They, however, also highlight the demanding nature of this model and propose a less demanding model, also distinguishing between random mating within patches and complete avoidance of sib-mating, that gives qualitatively similar response to LMC challenges. Under the 'Self Knowledge' model (assuming random mating), foundresses produce a sex ratio according to their fecundity relative to the average, giving similar predictions to the production of a constant number of males (Stubblefield & Seger, 1990).

At intermediate foundress densities, selection for 'Complete Knowledge' utilization and precise sex allocation is less strong than in species experiencing low foundress densities where the reward for taking into account clutch size differences is larger (Nagelkerke, 1996). It is thought that foundresses in species experiencing such foundress densities probably allocate sex with a binomial probability. The inevitable variation around the intended sex ratio, however, can lead to the production of too few sons. There is a strong selection pressure for producing enough sons to mate the females in the fig if foundresses do find themselves alone (Hamilton, 1967) and for these males to be able to chew an exit hole for the females to be released (Herre, 1989). It is thus expected that some kind of insurance in males will be selected for when clutch size is small to guard against the possibility that males might all die or no males will be produced (Green *et al*., 1982; Hardy, 1992, Nagelkerke, 1996), leaving females trapped (Greeff, 2002) or unmated West *et al.* (1997).

A strong negative correlation between clutch size and sex ratio has been reported in single foundress figs in several studies (Herre, 1987; West & Herre, 1998a; Kjellberg *et al.*, 2004). This behaviour further shows that it is possible that intermediate levels of information utilization can be effective in adaptation to LMC conditions. Foundresses allocating sex with binomial probability at intermediate foundress densities do not necessarily have lower fitness than an equivalent population of females allocating sex under a precise model of sex allocation coupled with the use of circumstantial information (Nagelkerke, 1996). Thus, while precise sex allocation is expected to out-compete binomial sex allocation in species producing small clutches and experiencing low foundress numbers, it is not expected that precise sex allocation will be typical in species that have large clutches and high foundress numbers.

In conclusion, these studies are all repaying the cautionary advice of Orzack and Sober (1994) that specific evidence is needed before one can assign causality, let alone claim generality of local adaptive optimality. All other influences on sex ratio being equal, LMC avoidance has been and still is considered a promising trait to study fine scale adaptation (Herre et el., 2001; Orzack, 2002). The task in fig wasps is thus to establish what information is being used under different specific scenarios and then to determine how it is used and if this agrees with theoretical predictions. Advances in genetic profiling techniques are making such studies easier by facilitating the resolution of relatedness of individuals within broods. Microsatellites are identified as being reliable for this purpose in hymenopterans (Queller *et al.*, 1993) and have been successfully used in fig wasp studies (Molbo *et al.*, 2003; Zavodna *et al.*, 2005). In the study that follows, this technology is used to investigate the behavioural response of the fig wasps *Platyscapa awekei* to clutch size variation in experimentally controlled two foundress figs.

Research Report

Introduction

Platyscapa awekei is the pollinator of the fig tree *Ficus salicifolia*. Foundress numbers are low in this species and it has been observed that contests over oviposition sites will often ensue between foundresses in two and three foundress figs (Moore & Greeff, 2002). In this work, it is recorded that during a contest, larger foundresses typically lift the smaller foundress from the surface of the fig while she lays her eggs. When the smaller foundress is released, she is able to lay her eggs. As a result of these contests, larger foundresses produce larger clutches while small foundresses are left to lay their eggs in the remaining sites that are available. It is thought that there will be a strong selective pressure for foundresses to use information on clutch size differences when allocating sex based on the outcome of contests because double foundress figs are frequently observed in this species and the resulting benefits of precise sex allocation will thus be high (Nagelkerke, 1996; Herre, 1987; West & Herre, 1998a).

Theory predicts that foundresses using information on own and other clutch size are expected to make the same absolute investment in males under the assumption of random mating within patches and that brood sex ratio gradually increases with an increase in brood size. Stubblefield and Seger (1990) refer to this model of information utilisation as ‘Complete Knowledge’. In the same publication, they also summarise a simpler model of information utilisation assuming random mating called ‘Self Knowledge’. Under this model, foundresses allocate sex according to their relative competitive ability within the population. Thus, smaller than average foundresses produce a sex ratio intermediate between the average population sex ratio and that predicted for the same foundress allocating sex under the model of ‘Complete Knowledge’. Similarly, large foundresses produce an intermediate sex ratio between the average population sex ratio and the same foundress allocating sex under the model of ‘Complete Knowledge’.

While the ‘Complete Knowledge’ model is likely to apply to a species like *P. awekei*, male dispersal has been observed (Greeff, 2002; Greeff *et al.* 2003). Male dispersal is expected to reduce the selective pressure for foundresses to produce precise sex ratios as excess males could disperse to look for a mate (Greeff, 2002; Moore *et al.*, 2005). There is a potential additional incentive for males to disperse to avoid inbreeding depression and

Local Mate Competition (LMC) (Greeff, 1996; Greeff & Ferguson, 1999; Hardy & Mayhew, 1998; Perrin & Mazalov, 2000; Moore *et al.*, 2005). While it is doubtful that inbreeding depression is likely to have a significant effect in a species with such high inbreeding levels, the possibility is investigated.

The relatively well documented life history of *P. awekei* thus provides opportunity to observe how individuals within a fig wasp species respond to adaptive challenges where their own species are taken to be part of the environment in a natural system where the parameters determining fitness are relatively clearly defined. Here, the sex allocation behaviour of *P. awekei* in double and single foundress broods collected from controlled introduction experiments is studied within the frame work of the 'Complete Knowledge' and 'Self Knowledge' models discussed above. Microsatellites were used to resolve maternity in double foundress broods in order to determine clutch size and sex ratio differences between foundresses and single foundress clutches were collected to serve as a control for non-contest induced influences on sex ratio.

The genetic data generated from double foundress broods identified instances of multiple mating and also provided information on maternal heterozygosity. In haplodiploids, males only pass on their genes to daughters. Females pass on genes through sons and daughters. Hence, multiply mated females might be influenced through seminal peptides to produce more female biased sex ratios than singly mated females. As females are expected to counter-respond, it is very unlikely that such an effect will be detected in nature. Nevertheless, this opportunity is taken to test this prediction. Similarly, the effect of maternal heterozygosity is tested for an effect on sex ratio. In systems with patchy mating, females either mate with brothers, or unrelated males. If there is an effect of inbreeding or outbreeding depression on female fitness, there may be an indirect effect on sex ratio through varying competitive abilities between foundresses of varying heterozygosity: weaker females will presumably produce smaller clutches. As this species has high inbreeding levels, an effect is not expected.

This information and information on clutch size difference was tested for their influence on sex ratio in a Generalised Linear Model (GLM) with binomial errors (Hardy, 2002; Crawley, 2005) and number of own sons was regressed against number of own daughters and number of other foundress sons and daughters in a GLM with Poisson errors. The latter allows a direct test of the prediction of the 'Complete Knowledge' model that foundresses use information of own and other foundress clutch size will make the same absolute investment in sons. Furthermore, monthly foundress counts were performed over the period of one year to build a temporal picture of variation in foundress number and to make an ecological estimate of inbreeding levels. These data were compared to genetic estimates of sib-mating made using the maternal genotype data from double foundress broods to determine male dispersal rates. Finally, observed mean absolute fitness of foundresses producing small and large clutches was compared using a standard inclusive fitness model for haplodiploids. The effect on average fitness of altering the sex ratio of each group while holding constant the other group is reported.

Materials and methods

Sample collection

Sampling was carried out at the National Botanical Gardens, Pretoria, South Africa during the summer of 2005/2006. Sampling was restricted to the summer months because Pretoria is at the edge of the distribution of the *F. salicifolia* and both tree and wasp activity ebbs during the relatively cold winters. Three *F. salicifolia* trees that produced reliable and comparable crops were used for the experiment (Tree 1, Tree 2 & Tree 3). 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. These figs were put into separate plastic tubes with gause tops. Female wasps that emerged that night were used the next morning for introductions.

Bags were taken off, one twig at a time, and great care was taken to ensure that only experimentally introduced wasps entered figs. Twigs were assigned for single or double introductions so as to be as evenly represented as possible with respect to size and appearance of the figs. In the case of double introductions, single wasps were first introduced into all of the figs on a twig. An attempt was 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, ensuring that foundresses were not related.

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 replaced securely.

Figure 1 Dispersing offspring 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.

When the un-bagged 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 gently fitted over it. As the fig expands, it seals the tube. Within a matter of days the fig reaches maturity and the wasps emerge (Figure 1). 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.

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 and produce sex ratios with binomial variation. 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. These included a second round of single foundress introductions on Tree 1 and of double foundress introductions on Tree2. Figs containing parasitic wasps had no effect on model predictions and were included in the analysis.

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 into separate 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 60ºC 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 55ºC 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 1 (I)]. PCR reactions were performed in an eppendorf Mastercycler® gradient machine in 96 well plates [Table 1 (II)]. 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.

Table 1 (I) 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

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 equally ambiguous with respect to maternity. In total, 11 females and 2 males were excluded from the 28 confirmed 2 foundress broods.

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. Family divisions were considered individually. When multiple paternity was unambiguous, foundress mating status was recorded as “TRUE” for multiply mated foundresses and “FALSE” for foundresses mated by one male.

Table 1 (II) 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
dNTPs	0.16mM
Taq DNA Polymerase	0.5U
Reaction volume	10 µl
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	0.5µl template
Buffer	1x
$MgCl_2$	pool 1: 2mM; pool 2: 1.8mM; pool 3: 2mM; pool 4: 1.5mM
Primers	0.3µM
dNTPs	0.16mM
Taq DNA Polymerase	0.5U
10 min., 95°C	10 µl
Reaction steps	**Conditions**
Hotstart	Reaction volume
Cycles	[95°C, 40 sec; pool*°C, 1 min.; 72°C, 2 min.] x 30 pool 4: 60°C pool 2: 65°C pool 3: 63°C pool 1: 50°C
Hold	4°C

Reaction pools	**Primer(s)**
1	Pa 1
2	Pa 4
3	Pa 7, Pa 8, Pa 21
4	Pa 32

Statistical analysis

Statistical analyses were performed in R 2.1.1.. In total, 28 double foundress broods (mean brood size = 88.89, range: 31-139; mean large clutch size = 49.18, range: 16-75; mean small clutch size = 39.71, range: 5-69) and 30 single foundress broods (mean brood size = 50.9, range: 9-77) were analysed. One female from each double foundress brood was randomly selected to be the subject representing that brood. The single foundress sample comprised 10 randomly selected single foundress broods from each tree. Five single foundress broods from Tree 1 and 8 from Tree 2 collected from double foundress introductions were also analysed. Single and double foundress broods were analysed separately.

Two categories of analyses were performed. GLMs with binomial errors were fitted using a logit link to analyse sex ratios (Wilson & Hardy, 2002; Crawley, 2005) and GLMs with Poisson errors were fitted using a log link for number of sons produced. Model simplification was carried out by stepwise deletion and successive models were compared using χ^2 tests. Minimum adequate models were retained and Tree was specified as a factor throughout analyses. When there was over-dispersion, quassibinomial/Poisson errors were specified and models were compared using F-tests (Crawley, 2005).

The GLMs fitted for the sex ratios of selected females from double foundress broods included own and other foundress clutch size, mating status (multiply mated: “TRUE” or “FLASE”) and number of heterozygous maternal loci as explanatory variables. Interaction terms between clutch size, mating status and maternal heterozygosity were tested. Clutch size was the only explanatory variable included in the GLM fitted for foundress sex ratios in single foundress broods and single foundress broods collected from double foundress introductions. Brood size was the only explanatory variable in the GLM fitted for brood sex ratio from double foundress introduction.

GLMs of number of sons produced for the selected females from double foundress broods were regressed against number of own daughters and number of other foundress daughters and other foundress sons. This model tested the prediction of the ‘Complete Knowledge’ model that foundresses using information of own and other clutch size make the same absolute investment in sons. Equivalent models were fitted for number of sons from single foundress introductions and for number of sons from the 13 single foundress broods collected from double foundress introductions. Total number of daughters was the only explanatory variable included in the GLM fitted for total number of sons form double foundress broods.

Ecological observations

Naturally occurring foundress numbers were observed from August 2005 to July 2006. One sample was collected from a fruiting tree every month (mean sample size = 177.75 figs, range: 50 to 200 figs). These included the 3 experimental trees. Sampling was performed once the receptive phase had ended. Figs were split in half and the number of dead foundresses counted under a dissecting microscope. Occasionally, foundresses appeared to be stuck in the bracts lining the ostiole of the fig. This was noticed most frequently in figs from trees with high foundress numbers. Stuck foundresses were included in the count. Figs containing live foundresses were not recorded as these figs were likely still receptive.

An ecological estimate of sib-mating was made from the foundress count data by taking the harmonic mean of the expected sib-mating for each category of foundress number under the assumption that mating is random and that sex ratios and clutch sizes are equal. An adjusted estimate of this value was also made using the sex ratios and clutch sizes observed in the experimental 2 foundress broods. The corresponding genetic estimate of sib-mating was taken using the allele frequencies recorded from the reconstructed maternal genotypes following Suzuki & Iwasa (1980): $f = \frac{s}{4-3s}$ where f is the inbreeding coefficient and s is the level of sib-mating. The difference between observed and estimated levels of sib-mating was taken as an estimate of male dispersal from the natal fig. The genetic estimate of sib-mating was used to calculate the predicted optimal sex ratio for haplodiploids (Taylor & Bulmer, 1980): $r^* = \frac{(n-1)(2n-1)}{n(4n-1)}$ where r^* is the unbeatable sex ratio and n is the harmonic mean foundress number. This is compared with the harmonic mean sex ratio across single and double foundress broods using foundress counts to weight the calculation.

Fitness calculations

Double foundress broods were divided into two groups: foundresses producing large clutches and foundresses producing small clutches. The absolute fitness of each foundress in the two groups, assuming random mating, was calculated using the inclusive fitness formula following Frank (1986): fitness = [*sons* / (*sons* + *other sons*)] x *relatedness to sons* x *reproductive value of sons* x [*daughters* + *other daughters*] + *daughters* x *relatedness to daughters* x *reproductive value of daughters* and the mean fitness of the two groups was compared. The fitness consequences were examined for replacing daughters with sons in incremental steps of 1 to 5 for each category of foundress, while keeping the clutch of the other category of foundress constant. The reciprocal calculation was performed where sons were replaced with daughters. In clutches where sons were exhausted, sons were left at 0 and no more daughters were subtracted.

Results

Sample collection

Experimental figs took between 1 and 2 months to develop from the date of introduction to the date of release. One twig from Tree 3 with 7 double foundress introductions was not collected after none of these figs had released by the time that all the figs on other twigs on the tree had been collected. These figs were badly infected by a fungus.

Of the collected broods, 35 broods contained non-pollinating wasps. It is unclear whether experimental figs were parasitized through the bag material, or before figs became receptive to pollinators. *Otitesella pseudoserata* was responsible for the majority of these cases; two broods containing *Philotrypesis sp.* were recorded. *O. pseudoserata* is a galling parasite, so its presence should not affect sex ratio directly. For this reason and sample size limitation, broods containing *O. pseudoserata* were not discarded. *Philotrypesis sp.* are parasitoids and have the potential to affect pollinator sex ratio by killing developing pollinator offspring (Cook & Rasplus, 2003). Only one brood containing *Philotrypesis* was included in the random sampling of double foundress broods that were later genotyped. Exclusion from the data set of this brood had no significant effect on model outcomes.

Maternity assignment

Discrepancies between manually assigned family groups and those assigned by COLONY were mostly minor. Two notable exceptions led to the exclusion from analysis of 1 brood from Tree 2 and 1 brood from Tree 3 as the possibility of presence of a third foundress could not be ruled out. 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 was that these two mothers were sisters. Allelic dropout provides a more reasonable explanation for the observation. The probability of 2 sisters entering the same fig under natural circumstances is very small, however the possibility could not be excluded given the nature of experimental introductions.

Statistical analysis

The minimum adequate GLM describing sex allocation of foundresses observed in double foundress broods [Figure 2 c] is predicted by the formula: $r = \frac{e^{-1.24-0.0448 own\_clutch+0.0377 other\_clutch}}{1+e^{-1.24-0.04485 own\_clutch+0.0377 other\_clutch}}$. Under this model, offspring sex ratios decrease with own clutch sizes (p = 0.000191) and increases with other foundress clutch sizes (p = 0.000267). When clutch sizes are equal, both foundresses are predicted to produce equivalent sex ratios. If clutch size differs, the foundress producing the larger clutch will produce a lower sex ratio than if clutch sizes were equal and the foundress producing the smaller clutch will produce a higher sex ratio than if the clutches were equal. [Table 2 (I) (a)]

One data point from Tree 2 was excluded for its influence on the model. This data point is represented by a clutch of 3 males and 2 females. Exclusion of this data point did not change the nature of model predictions. The exclusion of one clutch parasitized by *Philotrypesis* specie*s* also did not alter the nature of model predictions. None of the interaction terms between clutch size, mating status and maternal heterozygosity was statistically significant. A dispersion parameter of 1.469 was specified.

No trend was observed for the GLM of offspring sex ratio of single foundresses [Figure 2 b], but Tree effect was significant (p = 0.0194). The dispersion parameter for this model was specified as 1.423. [Table 2 I (b)]

Sex ratio decreased significantly with own clutch size (p = 0.0154) for single foundress broods collected from double foundress introductions [Figure 2 a]. The equation predicting sex ratio is: $r = \frac{e^{-0.482-0.0269 own\_clutch}}{1+e^{-0.482-0.0269 own\_clutch}}$. No Tree effect was observed. A dispersion parameter of 1.187 was specified. [Table 2 I (c)]

No trend was observed for the GLM of combined brood sex ratio from double foundress broods [Figure 2 d]. One data point was excluded from the model as it was an extreme outlier. A dispersion parameter of 1.172 was specified. [Table 2 (I) (d)].

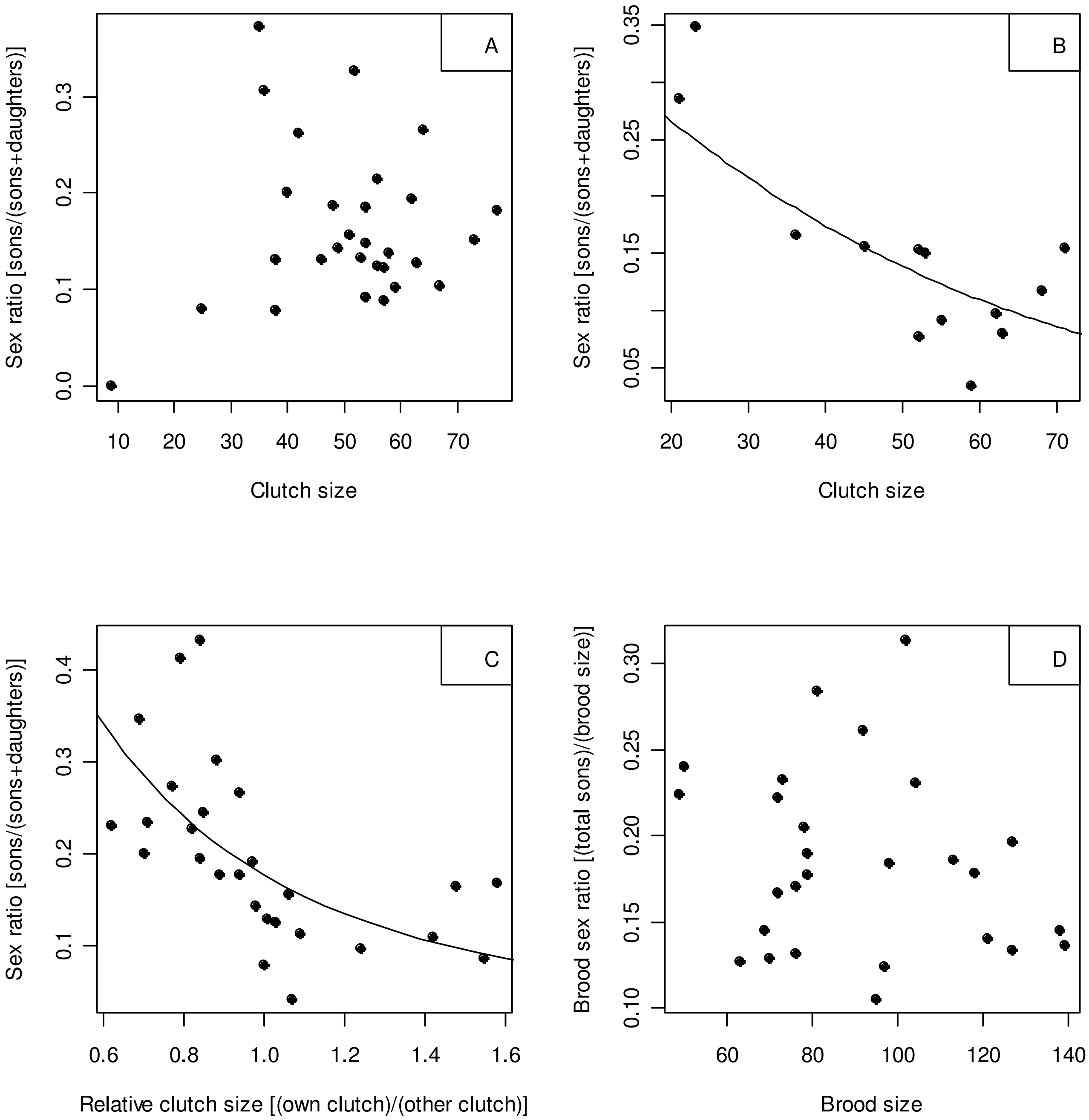

Figure 2 Sex ratio is shown for **A**: clutches from figs into which single foundress were introduced; **B**: single foundress clutches collected from double foundress introductions; and **C**: double foundress broods. **D**: brood sex ratio from double foundress figs is shown. Trend lines show model predictions where explanatory variables explained a significant component of the variation. A: clutch sex ratio shows no correlation with clutch size observed in single foundress broods (n = 30). B: Clutch sex ratio decreases as a function relative clutch size (n = 13). C: sex ratio decreases as a function of relative clutch size (n = 27). D: brood sex ratio shows no correlation with brood size in double foundress broods. (p 27)

Table 2 (I) Generalised Linear Models (GLM) of: (**a**) clutch sex ratio for 2 foundress broods; (**b**) 1 foundress broods; and (**c**) 1 foundress broods collected from figs into which 2 foundress were introduced, but were later revealed to be derived from one foundress following brood genotyping. **(d)** Brood sex ratio (total sons/ brood size) is modelled for double foundress broods. (p 28)

(a) GLM of foundress sex ratio in 2 foundress figs (n = 27)[†]

Parameters	**df**	**F**	**p**
Intercept	1, 24	11.929	0.00207**
Clutch size	1, 24	20.399	0.000142***
Other foundress clutch size	1, 24	18.731	0.000229***
Tree	2, 22	1.658	0.214
Maternal heterozygosity (# heterozygous loci out of 6)	1, 21	0.469	0.501
Multiple mating status (TRUE/FALSE)	1, 20	0.095	0.761

Dispersion parameter for quasibinomial family taken to be 1.469

(b) GLM of foundress sex ratio in 1 foundress figs (n = 30)

Parameters	**df**	**F**	**p**
Tree	2, 27	4.58	0.0194*
Clutch size	1, 26	0.268	0.609

Dispersion parameter for quasibinomial family taken to be 1.423

(c) GLM of foundress sex ratio in 1 foundress figs from double foundress introductions (n = 13)

Parameters	**df**	**F**	**p**
Intercept	1, 11	0.99	0.341
Clutch size	1, 11	8.2147	0.0154*
Tree	1, 10	0.0016	0.969

Dispersion parameter for quasibinomial family taken to be 1.187

(d) GLM of brood sex ratio of broods collected from double foundress introductions (n=27)

Parameters	**df**	**F**	**p**
Intercept	26	627.32	2e-16***
Brood	1, 23	0.637	0.433
Tree	2, 24	0.505	0.61

Dispersion parameter for quasibinomial family taken to be 1.720618

[†] Interaction terms between clutch, multiple mating status and maternal heterozygosity were considered. None was statistically significant.

The minimum adequate GLM for son production in clutches from double foundress broods is described by the formula: $sons = e^{1.53-0.0222own\_daughters+0.0338other\_daughters}$. Sons decreases with number of own daughters produced (p = 0.0003) and increases with an increase in number of other daughter produced (p = 1.601e-10). That is, the larger foundress is expected to produce fewer sons than expected for the average clutch for brood size experienced and the smaller foundress is expected to produce more sons than for the average clutch for brood size experienced. The same data point that was excluded in the GLM of sex ratio for foundresses ovipositing in double foundress broods was excluded from this model. The dispersion parameter was taken to be 1. [Table 2 (II) (a)]

No trend was observed for the minimum adequate GLM for number of sons produced for foundresses from single foundress figs, but Tree effect was significant (p<0.0194). A dispersion parameter of 1.643 was specified [Table 2 (II) (b)]. There was no significant correlation between number of sons produced and clutch size for the 13 single foundress broods collected from double foundress introductions. The dispersion parameter was taken to be 1 [Table 2 (II) (c)]. There was no significant correlation between total number of sons and total number of daughters from double foundress broods. A dispersion parameter of 2.0223 was specified [Table 2 (II) (d)].

Table 2 (II) Generalised Linear Models of: (**a**) number of sons for 2 foundress broods; (b) 1 foundress broods; and (**c**)1 foundress broods collected from figs into which 2 foundress were introduced, but were later revealed to be derived from one foundress following brood genotyping. (**d**) Total sons is modelled for 2 foundress broods. (p 29)

(a). GLM of son production in for double foundress figs (n = 27)

Parameters	**df**	**Δ deviance**	**p**
Intercept	1	36.073	1.9e-09***
Other daughters	1	40.901	1.601e-10***
Daughters	1	13.0021	0.0003***
Tree	2	1.4453	0.486
Other sons	1	0.0003	0.986

Dispersion parameter for poisson family taken to be 1

(b) GLM of son production in single foundress figs (n = 30)

Parameters	**df**	**F**	**p**
Tree	2, 27	3.782	0.0356*
Daughters	1, 26	2.756	0.1089

Dispersion parameter for quasipoisson family taken to be 1.643

(c) GLM of son production in single foundress figs from double foundress introductions (n = 13)

Parameters	**df**	**Δ deviance**	**p**
Intercept	1	171.47	3.54e-39***
Tree	1	0.024	0.877
Daughters	1	0.042	0.839

Dispersion parameter for poisson family taken to be 1

(d) GLM of total son production in double foundress broods (n = 28)

Parameters	**df**	**F**	**p**
Intercept	1	848.9	2e-16***
Tree	2	2.727	0.0849 .
Total Daughters	1	0.172	0.682

Dispersion parameter for quasipoisson family taken to be 2.0223

Ecological observations

The monthly foundress numbers measured August 2005 to July 2006 were combined. Single foundress figs were most common and represent the single category in which a foundress is most likely to oviposit. However, foundresses are more likely to oviposit in a multi-foundress fig than alone. The zero-truncated harmonic mean foundress number is 2.01 and foundress number ranged from 1 to 23 (Figure 3).

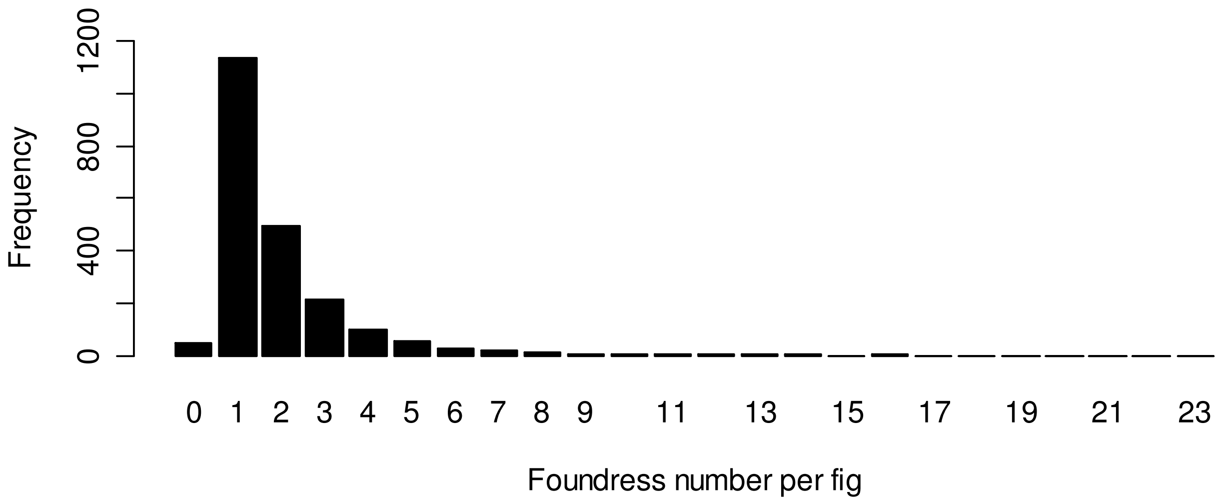

Figure 3 Monthly foundress counts were conducted for the period August 2005 - July 2006. The combined frequency distribution of foundress numbers per fig across the 12 samples (mean sample size = 177.75; ranged from 50 to 203) is given. Harmonic mean foundress number = 2.008.

The range and mode of foundress number within each month varied considerably. Single foundresses were observed at the highest frequency in 7 samples, including experimental Tree 2 and Tree 3. Double foundresses were observed at the highest frequency in 4 samples, including experimental Tree 1. Foundresses oviposited at the highest frequency in 4 foundress figs in 1 sample. Each distribution was zero truncated and compared with the predicted Poisson distribution using a χ^2 test (Table 3). Six of the 12 observed distributions showed no significant difference from expectation. The other 6 distributions showed a tendency for the category with the highest frequency of foundress number to exceed expectation, while categories on either side were under-represented relative to expectation.

Table 3 Number of foundresses per fig was counted from 12 collected samples spanning the year August 2005 to July 2006. Tree 1, Tree 2 and Tree 3 corresponds to samples collected in Sept., Oct. and Jan. respectively. Zero counts were excluded from the calculations. χ^2 comparisons of observed counts with predicted zero-truncated Poisson distributions for equivalent means are presented (observed|predicted). Counts with 4 and more foundresses were pooled. From these data, it appears that foundress entry into figs is more even than random, with the observed foundress distribution being clustered around the peak foundress density. (p 31)

Freq.	**Aug.**	**Sept.**	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**Jun.**	**Jul.**
0	29	0	5	4	0	1	0	2	0	0	5	0
1	166|166	59|71	172|171	26|35	103|52	162|165	87|87	67|60	19|10	63|72	176|175	35|34
2	8|8	83|64	20|22	38|24	52|59	35|30	61|65	68|61	23|23	79|64	17|18	11|12
3		36|38	3|2	8|12	19|45	2|4	40|32	27|42	37|35	35|38	2|1	3|3
4		15|17		4|4	4|26		6|12	17|22	41|39	13|17		1|1
5		5|6			3|12		6|3	11|9	23|34	8|6		
6		1|2			2|4			1|3	21|26	2|2		
7		1|-			4|1			3|1	12|16			
8					-|-			3|-	7|9			
9					2|-			1|-	5|5			
10					1|-				4|2			
11					2|-				4|1			
12					1|-				2|-			
13					1|-				1|-			
14					3|-				-|-			
15					0|-				-|-			
16					2|-				1|-			
17					0|-							
18					0|-							
19					0|-							
20					0|-							
21					0|-							
22					0|-							
23					1|-							
Total	174	200	195	76	200	199	200	198	200	200	195	50
μ	1.046	2.155	1.133	1.868	2.535	1.196	1.915	2.359	4.515	2.15	1.108	1.4
s^2	0.0862	24.882	0.332	1.658	140.606	0.359	2.07	11.146	58.609	3.179	0.261	1.298
χ^2	-	8.401	0.854	10.74	72.504	1.554	2.993	7.078	8.149	4.936	0.508	0.584
df	0	2	1	2	2	1	2	2	2	2	1	2
p	-	0.02*	0.4	0.005*	2.2e-16*	0.2	0.2	0.03*	0.02*	0.08*	0.5	0.7

The genetic and ecological estimates of sib-mating differ considerably. The ecological estimate of sib-mating is 0.72 when foundress clutch sizes and sex ratios are assumed equal and drops to 0.66 when sib-mating estimates observed in double foundress figs are used. This estimate is probably conservative as higher foundress number figs are also likely to have higher levels of outbreeding due to clutch size differences. The genetic estimate of sib-mating is 0.58. Thus, 8%-12% of matings are accounted for by male dispersal. Given the genetic estimates of sib-mating, the predicted optimal sex ratio for haplodiploids is estimated at 0.176.

The mean sex ratio observed across double and single foundress clutches of foundresses selected for analysis is 0.181. The mean sex ratio of foundresses selected for analysis from double foundress broods is 0.195 (median = 0.1808). The mean sex ratio for foundresses producing large clutches is 0.166 (median = 0.131) and the mean sex ratio for foundresses producing small clutches foundresses is 0.244 (median = 0.213). The mean sex ratio for single foundress broods is 0.160 (median = 0.140) and the mean sex ratio for single foundresses from double foundress figs is 0.147 (median = 0.151) (Figure 4).

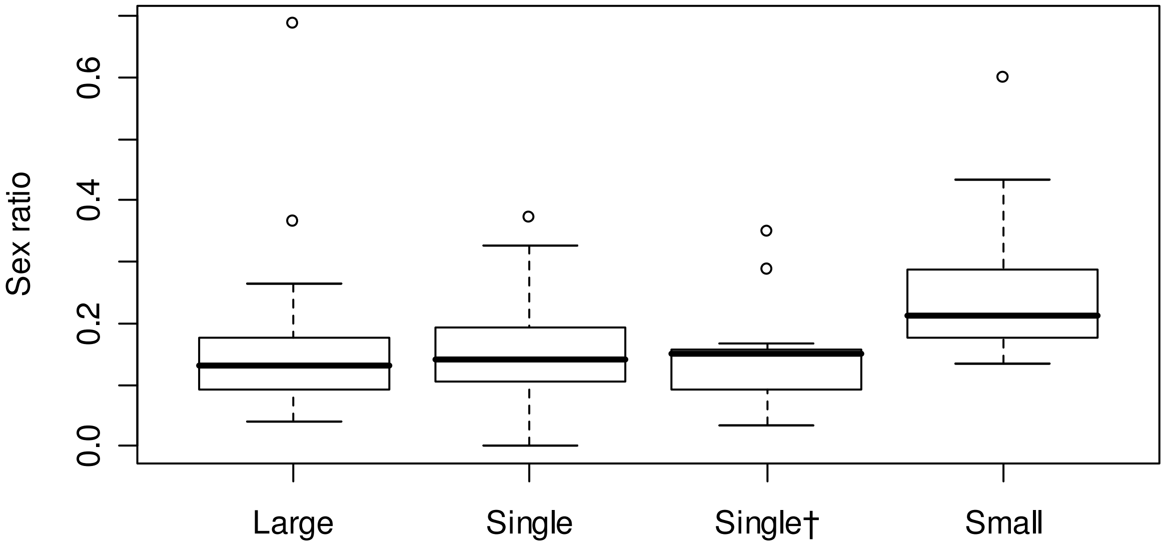

Figure 4 Sex ratio profiles are given for foundresses producing the larger clutches in double foundress broods (Large, n = 28); clutches from single foundress broods (Single, n = 28); clutches produced by single foundresses collected from double foundress introductions (Single[†], n = 13); and from foundresses producing the smaller clutches in double foundress broods (Small, n = 30). Boxes indicate the interquartile range of the data and whiskers extend beyond the boxes to the most distant datum within 1.5 times the interquartile range. Dots above the whiskers represent outliers falling outside of this range.

Fitness calculations

The mean fitness of foundresses producing the larger clutch in double foundress broods is 29.96 and that of females producing smaller clutch stands at 28.01. There is no significant difference in fitness between the means (p = 0.491; t=0.694, df=52.). An increase in sons in the larger clutches resulted in a slight mean increase in fitness for those clutches and a mean decrease in fitness of the smaller clutches [Figure 5 a]. A decrease in sons results in a steep decrease in fitness for those clutches and an increase in fitness of the smaller clutches. The mean fitness of smaller clutches is optimal, decreases slightly with the addition of more sons and decreases more steeply with the addition of more daughters. Fitness of foundresses producing large clutches decreases with the addition of sons and increases with the subtraction of sons [Figure 5 b].

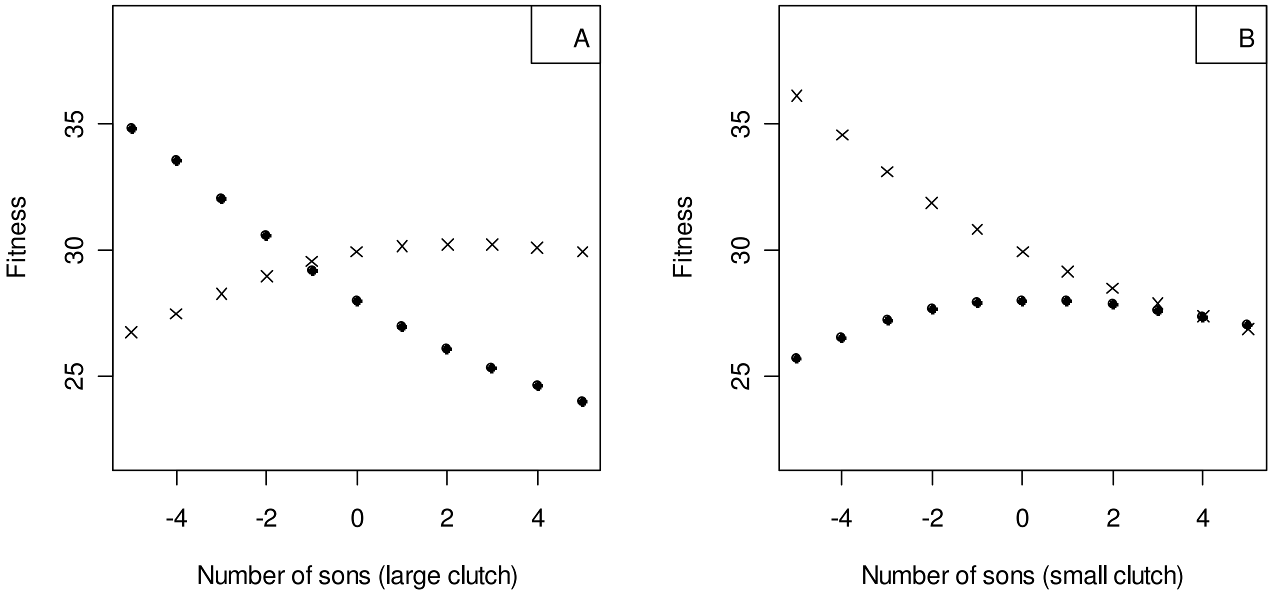

Figure 5 Average inclusive fitness profiles of large (x) and small foundresses (•) from double foundress broods are calculated by replacing observed number of sons with daughters in one group while keeping number of sons and daughters constant in the other group. **A**: number of sons of large foundresses is varied. The fitness of foundresses producing larger clutches increases with the addition of 1 and 2 sons, then decreases with the further addition of sons. Fitness decreases with the subtraction of sons. **B**: Number of sons of small foundresses is varied. Fitness is optimal for the observed sex ratio and decreases with the addition and subtraction of sons.

Discussion

The data collected here suggest that foundresses of the species *Platyscapa awekei* use information of clutch size differences caused by resource contest when allocating sex. Support for this claim comes from observation in double foundress broods that sex ratio decreases as a function of own clutch size and increases as a function of other foundress clutch size. Brood sex ratio shows a large range, so sex ratio is thus not determined solely as a function of clutch size. This is seconded by the observation that number of sons produced is determined as a decreasing function of number of daughters produced and an increasing function of the number of other foundress daughters produced. Foundresses are thus also not controlling sex by simply ovipositing a constant number of males. This is attested to by the relative consistency of brood sex ratio across brood size.

In compliment to this observation, the sex allocation behaviour of wasps ovipositing alone shows that foundresses are sensitive to foundress number: sex ratio is constant across clutch size in these broods rather than decreasing with clutch size, as expected under a model the 'Self-Knowledge' model (Stubblefield & Seger, 1990) where sex ratio is determined by clutch size alone. This response is clearly different from foundresses ovipositing in double foundress figs and to the behaviour of foundresses producing single foundress broods from double foundress introductions.

Further support for the recognition of foundress number comes from the ecological observation that foundress numbers tend to be highly clustered around the mean, suggesting that foundresses choose figs that have not been entered into when foundress density is high. While a Tree effect is detected in the analysed single foundress broods, it is not possible to interpret this finding with respect to foundress sensitivity to foundress number as samples from Tree 1 and Tree 2 were supplemented by a second round of collection for which foundress counts were not performed.

These findings agree qualitatively with the 'Complete Knowledge' model of Stubblefield and Seger (1990). The behaviour, however, does not fit predictions exactly. There is not a correlation between sons produced by foundresses producing large clutches and foundresses producing small clutches as predicted under the 'Complete Knowledge' model assuming random mating. Foundresses producing large clutches are in fact often observed to make a smaller absolute investment in sons than foundresses producing small clutches rather than making an equal investment in sons. Stubblefield and Seger (1990) propose a variation of the 'Complete Knowledge' model assuming avoidance of sib-mating. This model gives similar quantitative predictions to the observed data: sons are produced as a decreasing function of own clutch size and an increasing function of other foundress clutch size. Furthermore, this model also predicts that foundresses producing large clutches make a smaller absolute investment in sons than foundresses producing smaller clutches. It is suggested by a positive inbreeding co-efficient estimated from maternal genotypes that sib-mating does take place. Thus, this model cannot be applied here.

The fitness profiles suggest that foundresses producing small clutches are producing the optimal sex ratio given the sex ratio of foundresses producing large clutches. Foundresses producing large clutches, however, appear to produce too few sons. This is surprising because one would expect them to rather produce a few extra sons, as it has been proposed that males can always disperse (Greeff, 2002; Moore *et al.*, 2005). Furthermore, male dispersal is put as a means to avoid inbreeding depression (Greeff, 1996; Greeff & Furgeson, 1999; Hardy & Mayhew, 1998; Perrin & Mazalov, 2000). While we find no influence of female heterozygosity on fitness, male dispersal nevertheless could account for a decrease in expected inbreeding levels as estimated from the ecological observations.

Given that is appears as though foundresses directly use information of clutch size differences between own and other foundress clutch size in double foundress figs, it is interesting that data are overdispersed as this suggests that sex is allocated slightly more variably than the binomial expectation. Previous studies on *P. awekei* show that larger foundresses produce larger clutches. Assuming this to be so, a possible solution is that larger foundresses are laying eggs in high quality flowers. If there is a benefit to producing females in such flowers, one would expect large foundresses to take advantage of this opportunity (Trivers & Willard, 1973). This has been reported previously in hymenopterans (Werren, 1984, 1989), but there is no data collected here to investigate this further.

The observation that sex ratio is more female biased than predicted by theory has bearing on the work of Frank (1985). Frank pointed out that there will be more inbreeding in broods with clutch size differences if sex ratio is constant and that this could account for the systematic underestimation of population sex ratio by theory. At the same time, male dispersal will increase outbreeding levels and, in turn, the sex ratio through decreasing the relative genetic relatedness to daughters versus sons and the decrease in patch mating. This has been observed by West & Herre (1998b) in non-pollinating fig wasps.

Finally, it was investigated if there is an effect of multiple mating on sex ratio. The negative result we report was not unexpected. It is unclear, however, whether males have not evolved to influence female sex ratio or whether females have evolved a counter-response. This type of genomic conflict has been extensively studied, notably in *Drosophila,* where theory predicts a conflict of interests between male and female genes with respect to multiple mating. Males are expected to evolve means of forcing partners to lay all of their eggs after mating or to reduce attractiveness to other males. Experiments that allow males to evolve but not females have shown that this does happen and has been substantiated by the discovery of harmful proteins in the semen of *Drosophila* that accomplish this in a dose dependent manner with multiple mating (reviewed by Arnquvist & Lowe, 2005). The present study does not permit further speculation.

Considering the findings of this study, the accuracy of sex allocation remains disputable. The study nevertheless suggests that wasps use information about their own clutch size and other clutch size. This extends support for sex allocation theory applied to fig wasps in a species for which this behaviour is strongly selected and compliments the theoretical expectation of less refined methods of sex allocation in species where foundress density is higher and selection is not so strong for accurate sex allocation. The findings reported here are comparable to those reported by Kinoshita *et al.* (2002) and fall in place with the work showing that the level of adaptation observed is correlated with the frequency of encounter with the environment to which adaptation is expected (Herre, 1987; West & Herre, 1998a).

Conclusion

Studies on fig wasps join other studies on sex allocation behaviour in parasitic wasps, notably *N. vitripennis* (Suzuki and Iwasa, 1980; Werren, 1980, 1999; King, 1990; Flanagan *et al.*, 1993), as well as studies like those reported on aphids by Yamaguchi (1985). In all of these systems, similar behavioural adaptations have been recorded under conditions of high inbreeding and patchy mating. In all of these cases, the behaviour that has been observed exhibits some kind of harmony with the natural fluctuation observed within the system being studied. They show that the diversity of behaviour we see actually can be adaptive but that the nature of adaptive behaviour is determined by the environment being considered and is a function of the information that is available with respect to fitness, coupled with the means of response to use this information. Thus, more generally, diversity in intra-specific behaviour need not be incompatible with the notion of adaptation at the individual level, or at higher taxonomic levels, but in many cases may very well be seen to be its main source.

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