

The occurrence of sequential oviposition in fig wasps and the implications for interpreting sex ratio data

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Abstract

1. Pollinating fig wasps are believed to adjust their sex ratios according to standard local mate competition (LMC) theory.
2. Standard LMC theory assumes that all mothers ovipositing in a patch or fig does so simultaneously. However, it has been shown that fig wasps can oviposit sequentially.
3. We counted the number of figs containing dead and living mothers in figs where mothers entered naturally to estimate the incidence of sequential and simultaneous oviposition.
4. Single mothers were the norm in two wasp species, and multiple mothers the norm in the other two. However, contrary to LMC theory, in all four species, when multiple mothers occurred, sequential oviposition seems to occur more frequently than simultaneous oviposition.
5. The sex allocation problem fig wasp mothers face is thus more complicated than the widely assumed simultaneous ovipositing situation, and it leads to several expectations. Single mother's sex ratios should increase as the probability of additional mothers increases. Naturally founded multi-mother figs should have more female-biased sex ratios than the standard LMC model predicts for the final number of mothers. This is because early-arriving mothers underestimate the number of mothers and lay more daughters than the final number of mothers would require and later-arriving mothers can lay fewer sons to be competitive against the first mothers' too female-biased clutches.
6. Mothers must produce sex ratios that are optimised across a probabilistic range of foundress densities they experience.

KEYWORDS

fig wasp, local mate competition, oviposition, sequential, sex allocation, sex ratio

INTRODUCTION

Sex allocation theory aims to explain the observed maternal investment in sons and daughters (West, 2009). Hamilton (1967) illustrated

one of the most important of these, namely local mate competition (LMC). It applies when offspring of one or a few mothers forms an isolated mating population (=patch). He showed that a mother can reduce competition between her sons if she lays female-biased sex

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ratios. Taylor (1981) showed that these female-biased sex ratios also increase the number of mating opportunities for the mothers' sons. Herre (1985) further refined Hamilton's (1967) idea to give the evolutionary stable sex ratio for a mother in a group of n mothers as follows:

$$r_n^* = \frac{n-1}{n} \times \frac{1}{2} \times \frac{1+F}{1+2F}, \quad (1)$$

when the inbreeding coefficient is F . Although very convincing trends have been documented (West et al., 2000), core assumptions have only infrequently been tested (Hardy et al., 1999, 2000). In the almost 60 years following its derivation, LMC theory has grown with new ideas incorporated to give refinements such as Equations (1) and (2). Fig wasps have become one of the flagship examples in this field (West et al., 2000; but see Greeff & Kjellberg, 2022). This is in part because of the erroneous belief that their life history fits the assumptions of LMC models well and because an egg's sex is determined by if it is fertilised or not (Greeff & Kjellberg, 2022). In short, it is assumed that mothers (called foundresses) enter a fig in quick succession and lay their eggs simultaneously in the flowers inside the fig (the patch). Fig wasps follow the qualitative predictions of LMC theory, but the observed sex ratios of multi-foundress figs are too female-biased compared with the predictions (i.e., too few sons) (Greeff & Kjellberg, 2022).

One potential explanation could be if oviposition is sequential rather than simultaneous. Sequential oviposition is possible because figs remain receptive to wasps for several days after the first foundress entered (Khadari et al., 1995). Also, fig wasps can enter figs 24 h after the first foundress, when the first already died (Greeff & Compton, 1996; Wang et al., 2015). Furthermore, Kathuria et al. (1999) argued that differences in dead foundresses' egg loads suggest sequential oviposition in naturally founded figs. The optimal sex allocation for two sequential mothers (r_1^* and r_2^*) as well as the sum of their clutches (r_b^*) under LMC conditions is (Suzuki & Iwasa, 1980) as follows:

$$r_1^* = \frac{1+F}{1+2F} \times \frac{1+B}{2} \times \frac{p^2}{(1+p)^2}, \quad (2a)$$

$$r_2^* = \frac{1+F}{1+2F} \times \frac{1+B}{2B} \times \frac{p}{(1+p)^2}, \quad (2b)$$

$$r_b^* = \frac{1+F}{1+2F} \times \frac{1}{2} \times \frac{p}{1+p}, \quad (2c)$$

where B is the (second mother's number of offspring)/(first mother's number of offspring) and p is the frequency of patches with two mothers. The first mother should lay a more female-biased sex ratio than the second, whose optimal sex ratio is less biased or even male biased if its clutch is sufficiently smaller than that of the first. The predicted sex ratios increase with the incidence of patches with two mothers. The overall sequential sex ratio is predicted to be more female-biased than

Equation (1), except when there are always two females. This could explain why the observed sex ratios of naturally founded figs are significantly too female-biased (Greeff & Kjellberg, 2022).

Although sequential entry is certainly possible, not a single study has quantified its occurrence in natural populations. To address the assumption of simultaneous, as opposed to sequential oviposition in the field, we collected recently entered figs to quantify how frequently co-foundresses oviposit simultaneously versus sequentially.

METHODS

We collected figs from three individual trees of three *Ficus* species while the trees were receptive and wasps entered (Table 1). Batches of 50 figs were collected at a time and immediately prized in half to record the status of foundresses as alive, dead or moribund. Moribund was if a foundress was alive but had become uncoordinated. Collections were sometimes made several times on a single day. The ostiolar bracts were searched for foundresses that were trying to exit the fig. Wasps that died on entering were ignored while wasps that were alive and entering were counted as alive ($n = 2$).

From these values, we determined the number of figs that contained multiple foundresses and the frequency of sequential oviposition. For the latter, we only considered figs with at least one foundress that was alive or moribund. If all the foundresses were dead, it is impossible to distinguish between figs containing simultaneously and sequentially ovipositing wasps. We defined oviposition as simultaneous in all figs where all the foundresses were alive, sequential in any figs containing at least one living and one dead foundress and indeterminate in all figs containing moribund foundresses together with wasps that were either all alive or all dead as these may have oviposited mostly together with or not together with the other living or dead foundress(es). Given that the first foundress seems to only be able to change her sex ratio if the second foundress enters within 4 h of the first (Kinoshita et al., 2002), some cases scored as simultaneous may be functionally sequential. But, some cases we scored as sequential may be simultaneous because wasps of different ages entered simultaneously but did not die simultaneously.

The force of selection will depend on how frequently a foundress encounters a certain situation. By this logic, selection in a fig with two foundresses will be twice as strong as selection on a fig with one foundress. However, each foundress lays fewer eggs when there are more foundresses in a fig (Greeff & Newman, 2011; Wang et al., 2015), and the force of selection is thus in between the number of foundresses in a situation and the number of figs in a situation. Therefore, we counted the number of figs and calculated the number of foundresses affected by each situation.

RESULTS AND DISCUSSION

The frequency of multi-foundress figs varied substantially between the three pollinating species (Table 2, Figure S1). The non-pollinating,

TABLE 1 Details of collected species.

Ficus species	Fig wasp	Coordinates	Date range	Sample size
<i>Ficus ingens</i>	<i>Platyscapa soraria</i>	25°44'21" S 28°13'66" E	2023.02.23–2023.02.26	392 (515)
<i>F. salicifolia</i>	<i>P. awekei</i>	25°45'21" S 28°13'49" E	2022.03.31–2022.04.05 2023.08.02–2023.08.25	917 (936) 783 (795)
<i>F. sur</i>	<i>Ceratosolen capensis</i> <i>Sycophaga cyclostigma</i>	25°45'50" S 28°14'43" E	2023.05.02–2023.05.11	213 (745) 213 (3076)

Note: For each sample, the host tree, the wasp, the tree location, collection date and the sample size (the number of figs containing wasps and in brackets, the number of these wasps) are given.

TABLE 2 The numbers of figs, and of wasps, that were single foundresses, and the number of multi-foundress figs and wasps that contained simultaneously ovipositing wasps (sim: all foundress numbers greater than 1 with all wasps alive), sequentially (seq: figs with at least one foundress dead and one alive) and indeterminate (indet: figs containing a moribund female together with wasps that were either all alive or all dead; these wasps may have been alive with a now dead/alive female or may already have been moribund before the arrival of a living female).

Species	No. of figs		No. of figs with a live/moribund wasp		No. of wasps		No. of wasps with live/moribund wasp	
	Single	Sim	Indet	Seq	Single	Sim	Indet	Seq
<i>Platyscapa soraria</i>	290 (74)	4 (13)	11 (37)	15 (50)	290 (56)	9 (13)	26 (37)	35 (50)
<i>P. awekei</i> 2022	902 (99)	1 (9)	1 (9)	9 (82)	902 (97)	2 (8)	2 (8)	22 (85)
<i>P. awekei</i> 2023	771 (98)	0 (0)	1 (20)	4 (80)	771 (97)	0 (0)	2 (20)	8 (80)
<i>Ceratosolen capensis</i>	33 (15)	8 (20)	9 (23)	23 (58)	33 (4)	21 (12)	36 (21)	116 (67)
<i>Sycophaga cyclostigma</i>	4 (2)	32 (26)	5 (4)	86 (70)	4 (0)	381 (20)	84 (4)	1408 (75)

Note: Percentages are given in brackets after counts; for single a percentage of all figs and for sim, indet and seq a percentage of multi-foundress figs with at least one live or moribund individual. Number of wasps are given as well as number of figs because, for example, a two foundress fig is one instance of two foundresses but it affects two females.

but internally ovipositing species, *Sycophaga cyclostigma*, to which LMC should also apply, only had 2% of figs containing a single wasp (Table 2). Irrespective of how frequently multiple foundresses occurred, mothers from all four species tended to lay eggs sequentially, rather than simultaneously (Table 2). Therefore, these data suggest that sequential oviposition is the norm rather than the exception. Applying the standard LMC theory and looking at naturally founded figs is thus incorrect for two reasons. (1) In a single-foundress fig, the foundress has to hedge her bets on additional foundresses entering after she died by laying additional sons. (2) In multi-foundress figs, early-arriving foundresses will lay less sons than Equation (1) predicts for the final foundress number, and later-arriving foundresses should lay fewer sons because they lay eggs in figs containing too few males. Note that these conclusions remain the same if we count the number of foundresses rather than the number of figs (Table 2).

These data illustrate that single foundresses should not only lay just enough male eggs to fertilise and release all their mother's daughters but should also lay some extra males in case additional foundresses arrive later (Greeff & Compton, 1996). The increase in single foundress sex ratios of species with higher frequencies of multi-foundress figs supports this hypothesis (Greeff & Compton, 1996; Greeff & Kjellberg, 2022).

Although it seems reasonable to think that mothers can gather sufficient information to adjust their sex ratios facultatively in line with Equation (1), it seems improbable that sequential mothers from multi-foundress figs could gather and integrate sufficient information

to 'parameterise' Equation (2) or equivalent equations for higher foundress numbers. It seems more likely that females adopt a simple strategy that works well in most situations (Greeff & Kjellberg, 2022; Janetos & Cole, 1981). Such a strategy, supported by evidence, is that females tend to lay most of their male eggs first, followed by mostly female eggs (Greeff & Kjellberg, 2022). Then, smaller clutches, whether caused by many foundresses ovipositing simultaneously, or by a later female ovipositing sequentially, will result in less female-biased, or even male-biased, clutches. Although the resulting sex ratios are unlikely to be exactly what optimal theory would predict, given the female's relative number of offspring, her order in the sequence and the future expected arrivals, they will be qualitatively correct.

Models that can incorporate sequentiality, show promise (Chung et al., 2019; Moore et al., 2005). However, such models are analytically intractable and rely on numerical approaches to obtain optimal answers. The answers to this more simplistic question are thus more complex than Equation (1). The fact that Equation (1) predicts very similar answers to Equation (2) must not mislead us to believe that Equation (2) brings very little new insight and that the problem was basically understood before. Rather, we should appreciate that strongly female-biased sex ratios in fig wasps are the result of several forces: they reduce competition between brothers (Hamilton, 1967), they increase the number of matings of sons (Taylor, 1981), they allow more investment in more related daughters (Hamilton, 1979) and they

reflect the underestimates of foundress numbers by early-arriving foundresses that in turn require fewer sons from later-arriving foundresses to stay competitive.

AUTHOR CONTRIBUTIONS

Jaco M. Greeff: Conceptualization; investigation; funding acquisition; writing – original draft; writing – review and editing; formal analysis; project administration; data curation; supervision. **Simiso P. Mnguni:** Conceptualization; investigation; writing – original draft; writing – review and editing; formal analysis; data curation; project administration. **Tany Labuschagne:** Investigation; writing – review and editing; formal analysis. **Pamela J. de Waal:** Conceptualization; funding acquisition; writing – review and editing; supervision. **Simon van Noort:** Conceptualization; writing – review and editing; supervision; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All the counts on which this work is based and the calculations are available on figshare at [10.25403/UPresearchdata.24175050](https://doi.org/10.25403/UPresearchdata.24175050).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting Information.

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