

Virginity in haplodiploid populations: a comparison of estimation methods

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Abstract. 1. The presence of ovipositing virgin females, who are able to produce only male offspring, may have several important consequences for the evolution of reproductive strategies.

2. The prevalence of virginity for five fig wasp species was estimated using three different methods: (1) the proportion of females developing in fruit that contained no conspecific males; (2) dissection of females caught on sticky traps; and (3) dissection of females that had emerged from their galled flowers into the fruit cavity.

3. The estimates obtained by method 1 (females developing in single sex broods) were lower than those obtained by the other methods.

4. Across species, the estimates obtained by method 1 (females developing in single sex broods) were significantly correlated with those obtained by method 2 (sticky trap caught females).

5. Mating with sperm-depleted males is unlikely to occur in these species.

Key words. Fig wasps, parasitoid, sex ratio, sperm-depletion, virginity, wing dimorphism.

Introduction

In arrhenotokous haplodiploid animals, males develop from unfertilized eggs and females from fertilized eggs. Consequently, unmated (virgin) females are able to produce offspring, albeit only males. The presence of ovipositing virgin females may have several important consequences. These include influencing the evolution of mating systems (Hamilton, 1979; Godfray & Hardy, 1993; Greeff, 1995; Cook *et al.*, 1997), reducing the spread of parasitic chromosomes (Werren, 1987; Werren & Beukeboom, 1993), facilitating the evolution of eusociality (Godfray & Grafen, 1988), and selecting for mated females to adjust their offspring sex ratio (Godfray, 1990; Heimpel, 1994). Nonetheless, the possible role of virginity in all these processes depends ultimately upon the prevalence of virgin oviposition. This emphasizes the importance of obtaining reliable estimates of virginity levels in natural populations.

The most extensive studies into the importance of virginity have been carried out on fig wasps. Fig wasps develop in the

fruit of figs (*Ficus* spp.) and include both mutualistic pollinating species and parasitic non-pollinating species. Obtaining accurate estimates of virginity levels in fig wasps has been important for at least three reasons. First, a large number of species with very similar life histories can be studied easily, facilitating an examination of the ecological correlates of virginity levels (West *et al.*, 1997). Second, the factors influencing sex allocation in fig wasps are relatively well understood (Frank, 1985; Herre, 1985, 1987; Greeff, 1997; Herre *et al.*, 1997; West & Herre, 1998, in press), and so it has been possible to assess the consequences of virginity for sex allocation (West *et al.*, 1997). Third, estimates of virginity levels when females depart from their natal fruit are crucial to understanding the extreme cases of winged/wingless dimorphism that exist in male fig wasps (Hamilton, 1979; Greeff, 1995; Cook *et al.*, 1997; West *et al.*, 1997).

The main aim of this paper is to compare different methodologies for estimating the prevalence of virginity in fig wasps. Virginity levels are estimated by three methods for five fig wasp species associated with the African fig tree *Ficus burtt-davyi* Hutch. The secondary aim is to assess the role of mating by sperm-depleted males in leading to females constrained to produce only male offspring.

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Materials and methods

Natural history

Ficus burtt-davyi has a single species-specific pollinating wasp species, *Elizabethiella bajnathi*. A small number of pollen-bearing *E. bajnathi* females enter each receptive fruit, pollinate the uniovulate flowers within the fruit, probe some of the flowers with their ovipositors, and lay eggs into some of the ovaries (van Noort *et al.*, 1989; Compton, 1993). The offspring mature within the fruit, eating the contents of one flower each. Just before final ripening of the fruit, the wingless adult males chew their way out of the flowers in which they have developed. They then crawl around the interior of the fruit searching for flowers that contain female wasps. The males chew open these flowers and use their long telescopic gasters to mate with the females before they exit from the flowers. The females then emerge from the flowers and gather pollen, before leaving through a hole in the fruit wall chewed by the male wasps.

Four species-specific non-pollinating wasp species were also studied: two species that gall the female flowers (*Otitessella uluzi* Compton and *Otitessella sesquianellata* van Noort), and two parasitoid species (*Philotrypesis* sp. and *Sycoscapter* sp.). These non-pollinating species oviposit through the fig wall using highly elongated ovipositors. Adults of the non-pollinating wasps emerge from their galled flowers at approximately the same time as the pollinators and generally leave through the hole in the fruit wall chewed by the pollinating males. The males of these species were all wingless, and mating takes place within the cavity of the fruit, after females have emerged from their galls (Compton, 1993).

Estimating virginity method 1: females developing in single sex broods

The prevalence of virginity was estimated indirectly, as in previous studies, by determining the proportion of females developing in fruit with no conspecific males (Godfray, 1988; West *et al.*, 1997). Figs were sampled from the crops of four *F. burtt-davyi* trees in the Grahamstown area in 1989 and 1990. Mature fruit, without exit holes, containing wasps that would emerge the following morning, were collected, taken to a laboratory and cut open. The wasps that had already emerged from their galled flowers into the fruit cavity were removed and frozen. Later, the number and sex of each species of wasp that the fruit had contained were recorded, including those wasps still within galled ovules.

Estimating virginity method 2: dissection of females caught on sticky traps

The prevalence of virginity was estimated directly by determining the proportion of females caught on sticky traps whose spermathecae contained no sperm. Sticky traps were placed in the four trees sampled for method 1 as the wasps

were emerging. The traps were 21 × 30 cm sheets of clear plastic sprayed with pruning sealant. Females caught on these traps were dissected to see whether their spermathecae contained sperm (Hardy & Godfray, 1990). Individual females were removed from the trap, placed in a drop of water and squashed between two microscope slides. A compound microscope was then used to see whether the spermathecae contained any sperm.

In order to compare methods 1 and 2 a regression analysis was carried out, across species, on the estimates provided by each method. Data in Godfray (1988) provided two species from Papua New Guinea for this analysis: *Ceratosolen dentifer* Wiebes and *Philotrypesis* sp. For these two species, Godfray determined both the proportion of females developing in fruit containing no conspecific males (method 1) and the proportion of females exiting fruit that did not contain sperm in their spermathecae (similar to method 2). Analysis was carried out assuming binomially distributed errors in the GLIM statistical package, with the data rescaled by an appropriate Heterogeneity Factor (HF) to account for overdispersion (McCullagh & Nelder, 1983; Crawley, 1993).

Estimating virginity method 3: dissection of cavity-emerged females

The prevalence of virginity was estimated, in *E. bajnathi*, by determining the proportion of females that had emerged from their flowers into the fruit cavity that contained no sperm in their spermathecae. In *E. bajnathi*, mating takes place before females emerge from the galled flowers in which they develop (Compton, 1993). Consequently, a female that emerges into the fruit cavity as a virgin will remain so. The *E. bajnathi* females from method 1 that had already emerged from their flowers into the fruit cavity when the fruit was opened were used. These females were dissected to see whether their spermathecae contained sperm using the procedure outlined in method 2. This method could only be used for *E. bajnathi*, because in the other species males mate with females that have emerged into the fruit cavity.

Sperm depletion in males

The number of females that individual males had successfully mated was determined. For each species the females and males from method 1 were used, examining fruit in which only one male of that species had emerged from its galled flower into the fruit cavity. The females and males that had emerged into the cavity of these fruit were dissected to see whether their spermathecae or seminal vesicles contained sperm, using the procedure outlined in method 2.

Results

The number of fruits that contained each of the wasp species were 230, 108, 136, 183 and 192 for *E. bajnathi*, *O. uluzi*,

Table 1. Summary of the different virginity estimates. Wasp species is followed by the number of wasps and estimated percentage virgins for each of the three methods. Also shown is the G-test of the hypothesis that the proportion of virgins caught on sticky traps is equal to the proportion of females developing in fruit without conspecific males (* $P < 0.001$).

| Wasp species | Developing in single sex broods | | Caught on sticky traps | | Emerged into fruit cavity | | G-test |
|---------------------------------|---------------------------------|-----------|------------------------|-----------|---------------------------|-----------|--------|
| | Number | % Virgins | Number | % Virgins | Number | % Virgins | |
| <i>Elisabethiella baijnathi</i> | 5415 | 3.7 | 68 | 8.2 | 417 | 5.0 | 3.28 |
| <i>Otitesella oluzi</i> | 302 | 9.6 | – | – | – | – | – |
| <i>Otitesella sesquinellata</i> | 457 | 10.9 | 11 | 27.3 | – | – | 1.58 |
| <i>Philotrypesis sp.</i> | 788 | 4.1 | 61 | 6.6 | – | – | 0.68 |
| <i>Sycoscapter sp.</i> | 2000 | 2.1 | 51 | 15.7 | – | – | 16.11* |

O. sesquinellata, *Philotrypesis sp.* and *Sycoscapter sp.*, respectively. All species had female-biased sex ratios (defined as proportion of males): 0.16, 0.45, 0.42, 0.40 and 0.41, respectively.

Method 1. The proportion of females developing in fruit with no conspecific males is shown in Table 1 for each wasp species.

Method 2. Females of four species were caught on sticky traps. The numbers caught and the proportion whose spermathecae did not contain sperm are shown in Table 1.

Method 3. A total of 417 *E. baijnathi* females had emerged from their flowers into the fruit cavity when the fruit was opened. Of these, twenty-one (5.0%) lacked sperm in their spermathecae.

The estimates provided by method 2 (sticky trap-caught females) were higher than those from method 1 (females developing in single sex broods) for all four species in which estimates were obtained by both methods. This difference was, however, significant in only one species, *Sycoscapter sp.* (Table 1). The estimate provided by method 3 (cavity-emerged females) for *E. baijnathi* was intermediate between that obtained by the two other methods. This difference was not significant in either case (compared with method 1, $G = 1.66$, $P > 0.1$; compared with method 2, $G = 1.24$, $P > 0.1$).

Across species the estimate provided by method 2 (sticky trap females) was significantly correlated with that given by method 1 (females developing in single sex broods) ($\chi^2 = 4.88$, d.f. = 1, HF = 2.18; $n = 6$). Data for the two species collected by Godfray (1988) were included in this analysis ($n = 100$ in both cases): in both of these species no females developed in fruit without conspecific males, and 2% of females exiting fruit did not have sperm in their spermathecae.

None of the male fig wasps examined had empty seminal vesicles. The highest observed number of mated females in a fruit that contained one male was 21 ($n = 36$), 5 ($n = 32$), 4 ($n = 51$), 7 ($n = 63$), and 6 ($n = 25$) for *E. baijnathi*, *O. uluzi*, *O. sesquinellata*, *Philotrypesis sp.* and *Sycoscapter sp.*, respectively. This places a lower limit on the maximum number of females that an individual male can mate successfully. The average numbers of females developing per male in a fruit were 5.21, 1.22, 1.37, 1.49 and 1.40 for these species, respectively.

Discussion

Table 1 summarizes the estimates for the prevalence of virginity in the fig wasps of *F. burtt-davyi* using one indirect method

(the proportion of females developing in fruit that contain no conspecific males) and two direct methods (dissection of females caught on sticky traps; emerged from their galled flowers into the fruit cavity). The indirect method assumes that all females developing with conspecific males are mated, and that males do not become sperm-depleted. Dissection of females caught on sticky traps assumes that mated and unmated females disperse equally: if unmated females are slower to disperse from their natal tree, then virginity level may be overestimated with this method. For example, King (1993) showed that virgin females of the parasitoid *Nasonia vitripennis* fly less than mated females.

In all cases the direct methods gave higher estimates of the prevalence of virginity. This difference was statistically significant for one species, *Sycoscapter sp.* However, it should be noted that this may reflect limited statistical power, and that larger samples of sticky trap-caught females could have led to significance in more species. Across species, the proportion of virgins caught on sticky traps was significantly correlated with the proportion of females developing in fruit that contained no conspecific males. The results therefore suggest that, despite being an underestimate, the proportion of females developing in fruit that contained no conspecific males provides a reliable indicator of relative virginity levels when comparing across species.

The observation that some females that develop in fruit that contains conspecific wingless males do not mate successfully has implications for the evolution of mating systems in fig wasps. Some fig wasp species have dimorphic males: winged males that mate with females after dispersal from their natal fruit and wingless males that mate with females before they disperse. Hamilton (1979) suggested that this dimorphism could be maintained if the fitness of the two morphs was equal, and that the proportion of winged males in a species should therefore equal the proportion of females that develop in fruit without conspecific males. However, if some females developing in fruit containing conspecific wingless males do not mate successfully then the proportion of mates obtained by winged males may increase, thus favouring a higher proportion of winged males. Cook *et al.* (1997) have shown recently that in three out of nine fig wasp species with dimorphic males, the proportion of winged males was significantly greater than the proportion of females developing in fruit without conspecific wingless males.

One possible cause of constrained oviposition is having mated with a sperm-depleted male (Godfray, 1990). Considering fruit in which only one male of a species had emerged, there was never a sperm-depleted male. The highest number of mated females, in a fruit where only one male of that species had emerged, provides a lower limit on the maximum number of females that a male can mate successfully. These estimates varied from four to twenty-one across species and are all far greater than the average number of females developing per male in a fruit. These results suggest therefore that mating with sperm-depleted males is rarely likely to occur in the species examined here. Instead it suggests that the higher estimates of virginity levels given by the direct methods were due to some females not having mated, despite having developed in fruit containing males.

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