A comparative study of virginity in fig wasps

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Abstract. In haplodiploid species, the presence of unmated (virgin) females that can produce only haploid male offspring may have several important effects on a range of phenomena such as reproductive strategies, the transmission of parasitic chromosomes and the evolution of eusociality. The strength of these effects will depend upon the prevalence of virgin females. Two theories make conflicting predictions concerning the importance of factors that should be associated with increased levels of virginity, emphasizing either the degree of local mate competition or brood size. In this paper, a model is presented which predicts that, under conditions of local mate competition, the prevalence of virginity should be negatively correlated with the average number of offspring developing in a patch. The different predictions were then tested using data from 53 species of fig wasps representing 15 genera from four continents. Across species, the estimated prevalence of virginity was significantly inversely related to brood size, but showed no correlation with sex ratio (an index of local mate competition), supporting the predictions of our model. Qualitatively similar results were found when a formal comparative analysis was carried out using a morphologically and molecularly based phylogeny.

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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 selection on mated females to adjust their offspring sex ratio (Godfray 1990), reducing the spread of parasitic chromosomes (Werren 1987; Werren & Beukeboom 1993), influencing the evolution of mating systems (Hamilton 1979; Godfray & Hardy 1993) and facilitating the evolution of eusociality (Godfray & Grafen 1988).

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None the less, the possible role of virginity in all these processes depends ultimately upon the prevalence of virgin oviposition. This emphasizes the importance of estimating the prevalence of virginity in natural populations and identifying its correlates.

Godfray and Hardy (Godfray 1990; Hardy & Godfray 1990; Godfray & Hardy 1993) suggested that the prevalence of virginity should depend upon population structure. In subdivided populations, where the offspring of one or a few mothers mate amongst themselves in the natal patch, before the females disperse, a female-biased sex ratio (defined as proportion of males) is favoured by a process termed local mate competition (Hamilton 1967). Godfray and Hardy pointed out that selection penalizes virgin females heavily under local mate competition because they are unable to produce the favoured female-biased sex ratio, and so virgin females should be less common in species subject to local mate competition. To test this prediction, Godfray & Hardy (1993) used gregarious (high local mate competition) versus

solitary (low local mate competition) offspring development as a qualitative index of local mate competition and made an informal comparison across 24 species of Hymenoptera. However, the results contradicted their prediction: virginity was more prevalent in gregarious species than in solitary species.

In contrast, Heimpel (1994) demonstrated that high levels of virginity are expected in species where only one female oviposits in each patch (extreme local mate competition), clutches of eggs are small and there is appreciable developmental mortality. The difference in the two predictions arises because Godfray and Hardy implicitly assumed the absence of developmental mortality, which is estimated to range from 7 to 77% across the parasitic Hymenoptera (Nagelkerke & Hardy 1994). When only one female oviposits in a patch, local mate competition theory predicts an off-spring sex ratio of zero, which is usually inter-preted to imply production of just enough sons to ensure insemination of all the daughters (Hamilton 1967). However, developmental mor-tality leads to ovipositing females facing a trade-off between minimizing the number of sons produced, and insuring against the possibility that all the sons in a patch die, leaving virgin daughters (Green et al. 1982; Nagelkerke & Hardy 1994). Consequently, optimal sex allocation strategies can lead to appreciable levels of virginity under the population structure that has the most potential to penalize it (Heimpel 1994).

In the first part of this paper we extend Heimpel's model to demonstrate that appreciable levels of virginity are also expected under con-ditions of local mate competition when more than one female oviposits in a patch. Then, in the main part of the paper, we present new data estimating virginity levels in 47 species of fig wasps represent-ing 14 genera from three continents. Fig wasps develop in the fruit of figs (*Ficus* spp.) and include both mutualistic pollinating species and parasitic non-pollinating species. All of the pollinating and several of the non-pollinating species have wing-less males which are highly modified for mate location within the fruit (Hamilton 1979; Murray 1990). These males are unable to survive outside the fruit and so it can be assumed that any female leaving the fig unmated will remain unmated for the rest of her life (Godfray 1988). We consider only species with wingless males and estimate the prevalence of virginity by measuring the

proportion of females developing in fruits that contain no conspecific males.

We then use these and previously published data to test across fig wasp species the two main, and contradictory, predictions about the preva-lence of virginity. The first prediction (Godfray & Hardy 1993) is that virginity should be less preva-lent in species with high local mate competition. Several previous studies have suggested that the average sex ratios of both pollinating and non-pollinating fig wasps are likely to reflect the degree of local mate competition that they experience (Hamilton 1979; Frank 1985; Herre 1985, 1987; Herre et al., in press; S. A. West & E. A. Herre, unpublished data). Consequently, we use the aver-age sex ratio of a species as a surrogate measure of the degree of local mate competition to which it is subjected. If the first prediction is correct, we would expect a positive relationship, across species, between the proportion of females devel-oping in fruit that contain no conspecific males and average sex ratio. The second prediction (Heimpel 1994; this paper) is that virginity should be more common in species that are subject to local mate competition and have small average clutch sizes. If this second prediction is correct then we would expect a negative correlation between the proportion of females developing in fruti that contain no conspecific males and the average number of wasps developing in a fruit.

SMALL CLUTCH SIZES AND DEVELOPMENTAL MORTALITY

In this section and the Appendix we examine the influence of developmental mortality and clutch size on the optimal sex-ratio strategy, and result-ant level of virginity when two females oviposit in a patch. Our main aim is to examine whether developmental mortality can lead to appreciable levels of virginity when more than one female oviposits in a patch. We assume that two mated diploid females oviposit in each patch, and that each female lays a clutch of size M. The develop-mental mortality rate is d for males and D for females (see West & Godfray 1997 for how differ-ential mortality can affect the optimal sex-allocation strategy in panmictic populations). The assumptions of diploid genetics avoids the complications that arise from inbreeding in haplodiploids (Frank 1985; Herre 1985).

We assume that if a female develops in a patch in which at least one male survives, she is mated and has a relative fitness of one. If she develops in a patch without males her fitness is reduced by a factor v which reflects the costs of finding a mate or of remaining a virgin. We treat v as a constant although we are aware that this is a simplification. The fitness of a female developing in a fruit with no conspecific males will be zero (i.e. $v=1$) for the pollinating and non-pollinating fig wasp species where males are required to chew an exit hole in the fruit. The fitness return from an individual clutch will be the number of daughters produced, taking into account their mating status, and the total number of mates obtained by sons. The average fitness return from a clutch can be obtained by summing the fitness returns of all the possible brood compositions (e.g. one male dies, two males die, etc.) multiplied by the probability of their occurrence.

The model makes four predictions about sex-ratio strategies when two females oviposit in a patch (Fig. 1a). (1) Small clutch sizes permit only a limited set of brood sex ratios. For example, if a female lays only three eggs the sex ratio of her offspring can only be 0.33, 0.67 or 1 (one, two or three males, respectively). This may lead to disagreement with models of local mate competition which assume that any sex ratio can be produced (Nagelkerke 1996). (2) The optimum strategy is independent of female developmental mortality. (3) Increasing male mortality leads to selection for females to lay an extra son/sons (Fig. 1a). Extra sons are favoured to reduce the probability that all the males in a patch die, in which case the fitness return from a brood would be zero. (4) For certain values of male developmental mortality, at a given clutch size, the model predicts that mixed strategies may evolve. In such circumstances, each strategy has a higher fitness when in a patch with the other strategy, than when in a patch with a female who pursues the same strategy. This introduces a negative frequency dependence into the fitness of each strategy, which enables them to coexist.

The model makes three predictions about how virginity levels should vary when two females oviposit in a patch (Fig. 1b). (1) For a given clutch size the proportion of virgin females is expected to increase with rising male developmental mortality. This reflects an increased probability of all the males in a brood dying. (2) The proportion of virgin females is independent of female developmental mortality. (3) For a given male developmental mortality the proportion of virgin females is expected to increase with decreasing clutch size. Females who produce smaller clutches lay fewer males to produce the optimum sex ratio (Fig. 1a), and so all the males are more likely to die.

The predictions of our model are therefore similar to those involving only one female per patch (Heimpel 1994; Nagelkerke & Hardy 1994). In Fig. 1c,d we compare the predicted offspring sex ratio and prevalence of virginity in a case in which either one or two females lays the same number of offspring in a patch: a single female lays eight eggs, or two females each lay four eggs. The predictions for the two situations are very similar (in fact identical, for certain male mortality levels). Where the predictions differ, a single female ovipositing in a patch leads to a higher virginity level than two females ovipositing in a patch. This suggests that, for a given number of offspring developing in a patch, virginity levels may increase as the degree of local mate competition rises (in contrast to the predictions of Godfray & Hardy (1993)). If our model was extended to situations in which more than two females oviposited in a patch, we would expect to observe a similar pattern. These results suggest that, under conditions of local mate competition, the average number of offspring developing in a patch is likely to be a far better predictor of virginity levels than the number of females that oviposit in a patch.

FIG WASP NATURAL HISTORY

Fig wasps develop in the fruit of figs and include both mutualistic pollinating species and parasitic non-pollinating species. The fig trees are completely dependent upon the pollinators for the dispersal of pollen between fruit. In contrast, the non-pollinators provide no apparent benefit to the fig. The pollinating wasps are all members of the subfamily Agaoninae in the chalcidoid family Agaonidae, and have relatively similar life cycles. Each fig species usually has a single species-specific pollinating wasp species (Ramirez 1970; Wiebes 1979; Herre et al. 1996). A small number of pollen-bearing females (foundresses) enter each receptive fruit, pollinate the uniovulate flowers within the fruit, probe some of the flowers with
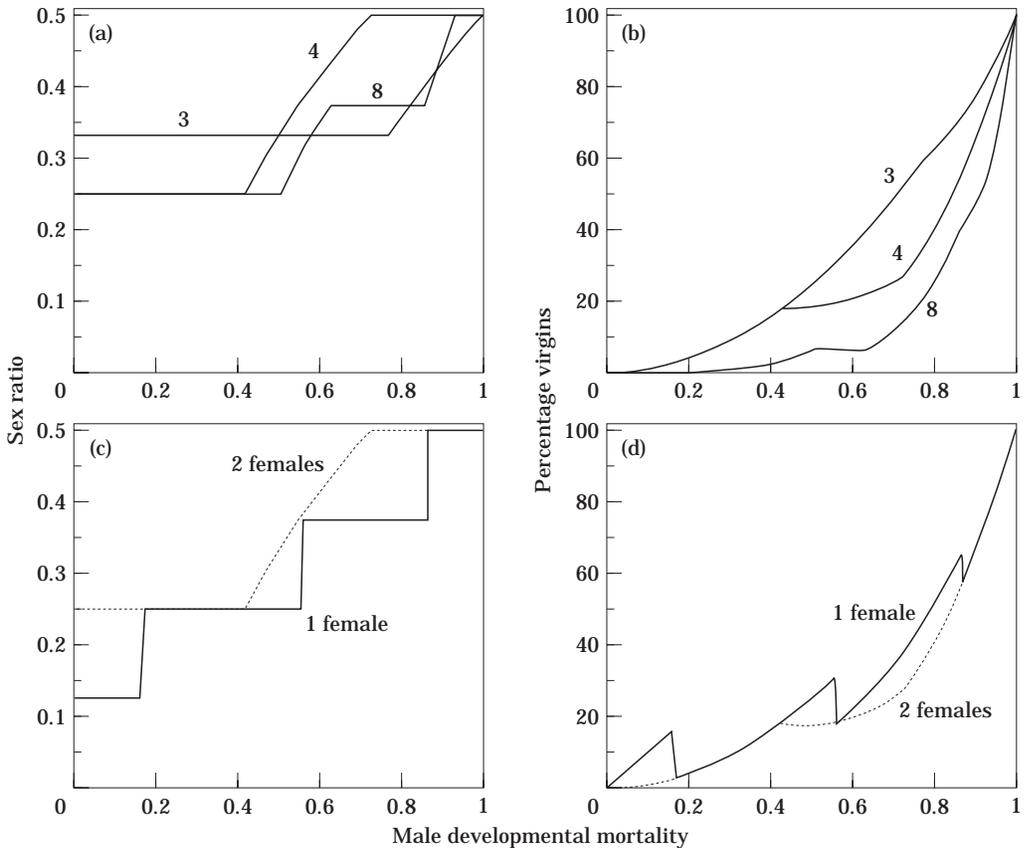
Figure 1. (a,b) The optimal sex ratio (a; proportion of males) and resultant prevalence of virginity (b) when two females oviposit in a patch. The different lines represent situations when both females lay a clutch size of three, four or eight. In all cases the fitness of a virgin female is assumed to be zero (i.e. $v=1$). (c,d) The predicted offspring sex ratio (c) and prevalence of virginity (d) in a case in which either one or two females produces the same total brood size: a single female lays eight eggs, or two females each lay four eggs.

their ovipositors and attempt to lay eggs in the ovaries (Frank 1984; Herre 1989; van Noort et al. 1989). These foundress wasps are usually unable to leave the fruit and subsequently die, having produced only one brood in their entire life. The offspring mature within the fruit eating the contents of one flower each (Herre 1989, 1996; Bronstein 1992). Just before the fruit ripen fully, 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 mate with the females. The females then emerge from their flowers and gather pollen, before leaving through a hole in the fruit wall chewed by the male wasps (Herre 1989).

The non-pollinating wasps also appear to be generally restricted to a single host fig species (Boucek 1988, 1993; Machado et al. 1996). However, a single fig species may have a large number of associated non-pollinating wasps. For example, 29 species of non-pollinating wasps have been recorded from one Old World fig species (Compton & Hawkins 1992). The majority of the non-pollinating wasp species belong to several other subfamilies of the Agaonidae and show an enormous range of life cycles (Boucek 1988; West et al. 1996). Some species develop within female flowers in a similar way to the pollinating wasps, others develop within large induced galls, and some are parasitoids (Godfray 1988; Bronstein 1991; West & Herre 1994; Kerdelhue & Rasplus 1996; West et al. 1996). Unlike the pollinating

wasps, the females of most non-pollinating species, and all the species considered in this paper, do not enter the fruit in order to lay their eggs. Instead females oviposit into a fruit from the outside. The adult non-pollinating wasps emerge from their 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 adult males of the non-pollinating wasps show an enormous diversity in morphology and behaviour (Hamilton 1979; Frank 1987; Murray 1987, 1989, 1990; Cook et al. 1997). While some species produce wingless males, as the pollinators do, others produce winged males that are able to mate away from their natal fruit. Finally, some species produce both wingless and winged males.

METHODS

We sampled naturally occurring fig species in Australia, Panama and South Africa. Detailed collection methods for the various species are given elsewhere (Herre 1989; Compton 1993; West & Herre 1994; Cook & Power 1996). However, the general pattern was that fruit development was monitored on several trees. We visited any trees bearing a fruit crop near maturation more frequently and then collected a sample of 40–50 figs before any wasps had exited the fruit. We then cut open each fruit and all the wasps were allowed to emerge before being frozen or stored in alcohol. Later, we recorded the sex and species of each wasp that the fruit had contained. We were then able to determine the proportion of females that developed in fruit that contained no conspecific males, the average total brood size and the overall sex ratio for each species. We used the proportion of females developing in fruit without conspecific males as an estimate of the prevalence of virginity (Godfray 1988).

In addition, we obtained published data on the prevalence of virginity for eight more fig wasp species with wingless males. Godfray (1988) estimated the prevalence of virginity in four species from Papua New Guinea: for two species by examining the spermathecae of females collected exiting fruit, and for two other species by calculating the proportion of females developing in fruit without conspecific males, as described above. Hamilton (1979) recorded the proportion

of females developing in fruit that contained no conspecific males for four Brazilian fig wasp species with wingless males (probably from the genus *Pegoscapus* and not *Blastophaga* as Hamilton stated).

STATISTICAL ANALYSIS

The data were analysed by two different methods. First, we used species as independent data points. Second, we used the method of independent comparisons to carry out a formal comparative analysis (Felsenstein 1985; Burt 1989). For each of these methods we examined the data for the pollinator and the non-pollinator species separately, as well as the pooled data set.

Species as Data Points

To avoid the problems of non-normal errors and unequal sample sizes that beset proportion data, we first analysed the data with a weighted general linear model analysis of deviance, assuming binomial errors in the GLIM statistical package (Crawley 1993). The number of females developing in fruit that contained no conspecific males was used as the response variable and the total number of females of that species as the binomial denominator. The inverse of total number of wasps, of that species, developing in a fruit (hereafter termed total brood size) and the arcsine square root of sex ratio were used as explanatory variables. However, after fitting the explanatory variables, the ratio of Pearson's chi-square to the degrees of freedom was over 30, showing considerable overdispersion. This showed that the data did not fit the assumption of binomial errors (McCullagh & Nelder 1983). Consequently, the proportion of virgin females was arcsine square-root transformed and used as the response variable in a multiple regression analysis assuming normal errors. In all subsequent analyses the residuals showed no obvious pattern, and plots of the ranked residuals against the standard normal deviates were reasonably straight lines. This suggests that the assumption of normal errors was appropriate.

Formal Comparative Analysis

Species may not be independent data points because they are phylogenetically related
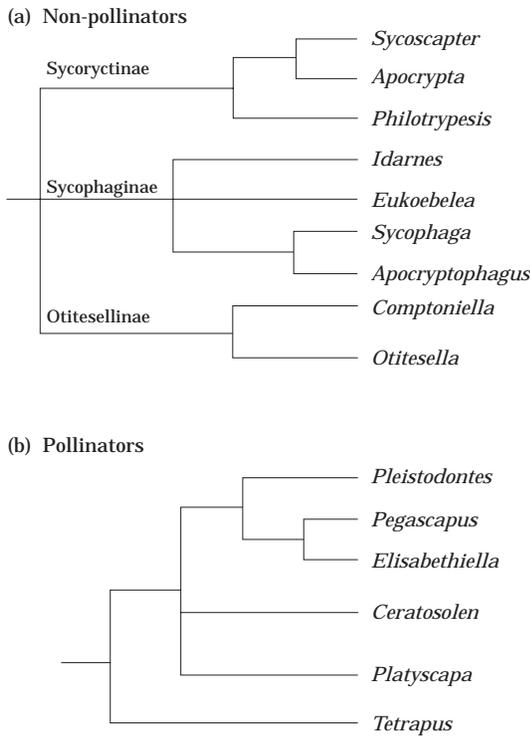
(a) Non-pollinators

(b) Pollinators

Figure 2. The phylogenies used in our formal comparative analyses. Branch lengths are arbitrary.

(Felsenstein 1985). This leads to problems if a trait is evolutionarily conserved, or highly correlated with an unknown phylogenetically inert third variable. Modern comparative methods are able to circumvent this problem by using the phylogeny of the species being examined to construct a series of independent contrasts. Independent contrasts are derived by comparing pairs of species or higher nodes that share a common ancestor (Felsenstein 1985). Grafen (1989) and Pagel (1992) have each provided methods for calculating a set of independent contrasts when the phylogeny is not perfectly resolved. Hypothesis testing can then be carried out on these contrasts with a regression fixed through the origin or a sign test (Harvey & Pagel 1991).

In Fig. 2 we present the phylogeny that we have used in our analyses. The non-pollinator and the pollinator wasps are separated into two distinct branches of a monophyletic group following the molecular phylogeny of Machado et al. (1996). The non-pollinator species are divided into three main subfamilies (Otitesellinae, Sycophaginae and Sycoryctinae) following the taxonomic groupings of Boucek (1988, 1993). The placing of the genera within the Sycorytinae follows the molecular phylogeny constructed by Machado et al. (1996). The *Apocryptophagus* and the *Sycophaga* are placed together in the Sycophaginae following Boucek (1988). The placing of the pollinator genera follows the molecular phylogeny constructed by Herre et al. (1996). *Platyscapa* are included in this following Wiebes (1982). Where there was no clear indication of branching order, a conservative approach was adopted with nodes left as polychotomies.

Given this phylogeny we are able to examine the relationship between the proportion of females developing in fruit that contain no conspecific males, total brood size and sex ratio using the method of independent contrasts. All the variables were transformed prior to analysis: the inverse of total brood size was used instead of total brood size, while the proportion of females developing in fruit that contain no conspecific males and the sex ratio were both arcsine square-root transformed. Contrasts (i.e. differences) for sex ratio (hereafter termed sex-ratio contrasts) and proportion of females developing in fruit that contain no males (hereafter termed estimated virgin contrasts) were derived using the Turbo Pascal program *Comparative Analysis by Independent Contrasts* (Purvis & Rambaut 1995). This package enables both the Grafen (1989) and the Pagel (1992) methods for calculating branch lengths to be used.

RESULTS

The proportion of females developing in fruit that contained no conspecific males varied from 0 to 35.3%. Table I shows the estimates for the non-pollinating species and Table II those for the pollinating species.

Species as Data Points

The proportion of females developing in fruit that contained no conspecific males (our estimate of the virginity level) was significantly positively correlated with 1/total brood size (Fig. 3a; all species: $F_{1,51}=84.64$, $P<0.01$, $r^2=0.62$, $N=53$; pollinator species only: $F_{1,19}=81.33$, $P<0.01$, $r^2=0.81$, $N=21$; non-pollinator species only:

Table I. Characteristics of the non-pollinating wasp species

Fig species Ficus	Wasp species	Sample size	Total brood size	Average sex ratio	Estimated % virgin females
Australia					
macrophylla	Sycoscapter australis	46	31.7	0.511	1.4
rubiginosa	Eukoebelea sp.	4	7.0	0.393	5.9
rubiginosa	Philotrypesis sp.	33	3.2	0.375	32.3
rubiginosa	Sycoscapter sp.	84	12.5	0.462	5.3
Brazil					
species 1	Idarnes sp.	12	50.2	0.281	0.0
species 2	Idarnes sp.	57	51.7	0.355	0.0
Panama					
bullenei	Idarnes sp.	6	8.8	0.132	13.0
citrifolia	Idarnes sp.	72	20.0	0.206	0.2
columbrinae	Idarnes sp.	4	9.8	0.256	0.0
costaricana	Idarnes sp.	8	30.6	0.273	0.0
dugandi	Idarnes sp.	46	22.5	0.221	1.6
obtusifolia	Idarnes sp.	18	53.6	0.310	0.0
perforata	Idarnes sp.	3	15.0	0.156	0.0
popenoei	Idarnes sp.	21	100.3	0.330	0.0
trigonata	Idarnes sp.	12	126.4	0.347	0.4
Papua New Guinea					
hispidioides	Apocrypta mega	46	4.9	0.319	23.4
hispidioides	Apocryptophagus sp.	98	15.0	0.260	4.0
hispidioides	Philotrypesis sp.	12	0–100	0.340	2.0
South Africa					
burtt-davyi	Otitesella uluzi	108	5.1	0.450	9.6
burtt-davyi	Otitesella sesquianellata	136	5.8	0.423	11.1
burtt-davyi	Philotrypesis sp.	183	7.2	0.402	7.9
burtt-davyi	Sycorytes sp.	122	17.9	0.417	2.0
cordata cordata	Comptoniella sp.	16	6.5	0.413	6.6
cordata cordata	Sycorytes sp. (pale)	13	5.3	0.362	2.3
cordata cordata	Sycorytes sp. (dark)	4	6.8	0.185	13.6
cordata salcifolia	Philotrypesis sp.	13	7.7	0.280	18.1
cordata salcifolia	Sycorytes sp.	20	8.7	0.225	6.7
sur	Apocrypta guineensis	45	94.6	0.562	0.1
sur	Sycophaga cyclostigma	20	92.7	0.223	0.0
thoningii	Otitesella tsamavi	39	16.9	0.441	1.4
thoningii	Sycorytes sp.	33	8.6	0.358	3.3
verruculosa	Philotrypesis sp.	20	21.4	0.391	2.3
verruculosa	Sycorytes sp.	8	9.3	0.108	15.2

Each *Ficus* species is followed by associated wasp species, the total number of fruit sampled, total brood size (average number of wasps, of that species, developing in a fruit), overall sex ratio, and the estimated percentage of virgin females (proportion of females developing in fruit containing no conspecific males). The data for the Brazilian and the Papua New Guinean species come from Hamilton (1979) and Godfray (1988), respectively.

$F_{1,30} = 70.90$, $P < 0.01$, $r^2 = 0.70$, $N = 32$). In contrast, there was no significant relationship between the proportion of females developing in fruit that contained no conspecific males and the sex ratio of a species (Fig. 3b; all species: $F_{1,50} = 2.95$, NS, $N = 53$; pollinator species only: $F_{1,18} = 0.18$, NS, $N = 21$; non-pollinator species only: $F_{1,29} = 0.61$, NS, $N = 32$).

Formal Comparative Analysis

The phylogeny shown in Fig. 2 allowed the calculation of nine independent contrasts within the pollinator species, 11 independent contrasts within the non-pollinator species, and one independent contrast between the pollinator and the non-pollinator species. Independent contrasts

Table II. Characteristics of the pollinating wasp species

Fig species *Ficus*	Wasp species	Sample size	Total brood size	Average sex ratio	Estimated % virgin females
Australia					
macrophylla	*Pleistodontes froggatti*	48	74.9	0.343	0.0
rubiginosa	*Pleistodontes imperialis*	128	87.5	0.336	0.8
Brazil					
species 1	*Pegoscapus* sp.	11	115.1	0.070	0.0
species 2	*Pegoscapus* sp.	55	60.6	0.091	2.4
Panama					
bullenei	*Pegoscapus tonduzi*	5	155.9	0.069	0.0
citrifolia	*Pegoscapus orozcoi*	57	133.1	0.068	0.0
columbrinae	*Pegoscapus esterae*	4	38.5	0.110	0.0
costaricana	*Pegoscapus* sp.	15	121.9	0.129	0.0
dugandi	*Pegoscapus* sp.	59	103.8	0.093	1.2
maxima	*Tetrapus* sp.	4	186.0	0.094	0.0
obtusifolia	*Pegoscapus hoffmeyeri*	13	268.3	0.120	0.0
perforata	*Pegoscapus insularis*	4	63.8	0.063	0.0
popenoei	*Pegoscapus gemellus*	38	228.3	0.177	0.0
trigonata	*Pegoscapus grandi*	6	559.0	0.352	0.0
yoponensis	*Tetrapus* sp.	22	137.1	0.205	0.0
Papua New Guinea					
hispidioides	*Ceratosolen dentifer*	10	1000–3000	0.390	2.0
South Africa					
burtt-davyi	*Elisabethiella baijnathi*	230	28.1	0.161	3.7
cordata cordata	*Platyscapa* sp.	9	8.8	0.139	35.3
cordata salcifolia	*Platyscapa* sp.	23	18.6	0.290	4.3
sur	*Ceratosolen capensis*	48	217.1	0.270	0.0
thoningii	*Elisabethiella* sp.	52	62.5	0.133	1.0
verruculosa	*Platyscapa* sp.	38	63.8	0.073	8.9

See Table I legend for details.

were calculated using both the Grafen (1989) and the Pagel (1992) methods for calculating branch lengths.

In all possible analyses a regression fixed through the origin showed a significant positive relationship between the estimated virgin contrasts and the 1/total brood size contrasts (Fig. 3c; all species: Grafen method, $F_{1,20}=132.19$, $P<0.01$, $N=21$; Pagel method, $F_{1,20}=74.37$, $P<0.01$, $N=21$; pollinator species only: Grafen method, $F_{1,8}=55.49$, $P<0.01$, $N=9$; Pagel method, $F_{1,8}=82.24$, $P<0.01$, $N=9$; non-pollinator species only: Grafen method, $F_{1,10}=137.47$, $P<0.01$, $N=11$; Pagel method, $F_{1,10}=132.65$, $P<0.01$, $N=11$).

In none of the analyses was there a significant relationship between the estimated virgin contrasts and the sex-ratio contrasts (Fig. 3d; all species: Grafen method, $F_{1,19}=0.09$, NS, $N=21$; Pagel method, $F_{1,19}=0.49$, NS, $N=21$; pollinator species only: Grafen method, $F_{1,7}=0.03$, NS, $N=10$; Pagel method, $F_{1,7}=0.10$, NS, $N=10$; non-pollinator species only: Grafen method, $F_{1,9}=0.48$, NS, $N=11$; Pagel method, $F_{1,9}=0.14$, NS, $N=11$).

DISCUSSION

Heimpel (1994) showed theoretically that when only one female oviposits in a patch, the proportion of virgin females increases with both (1) increasing male developmental mortality and (2) decreasing clutch size. Empirical support for these predictions comes from recent studies of intraspecific variation in two different bethylid wasps (Morgan & Cook 1994; Hardy & Cook 1995). Our model extends Heimpel's to the case where two females oviposit in each patch. The predictions for the two female case are similar to the
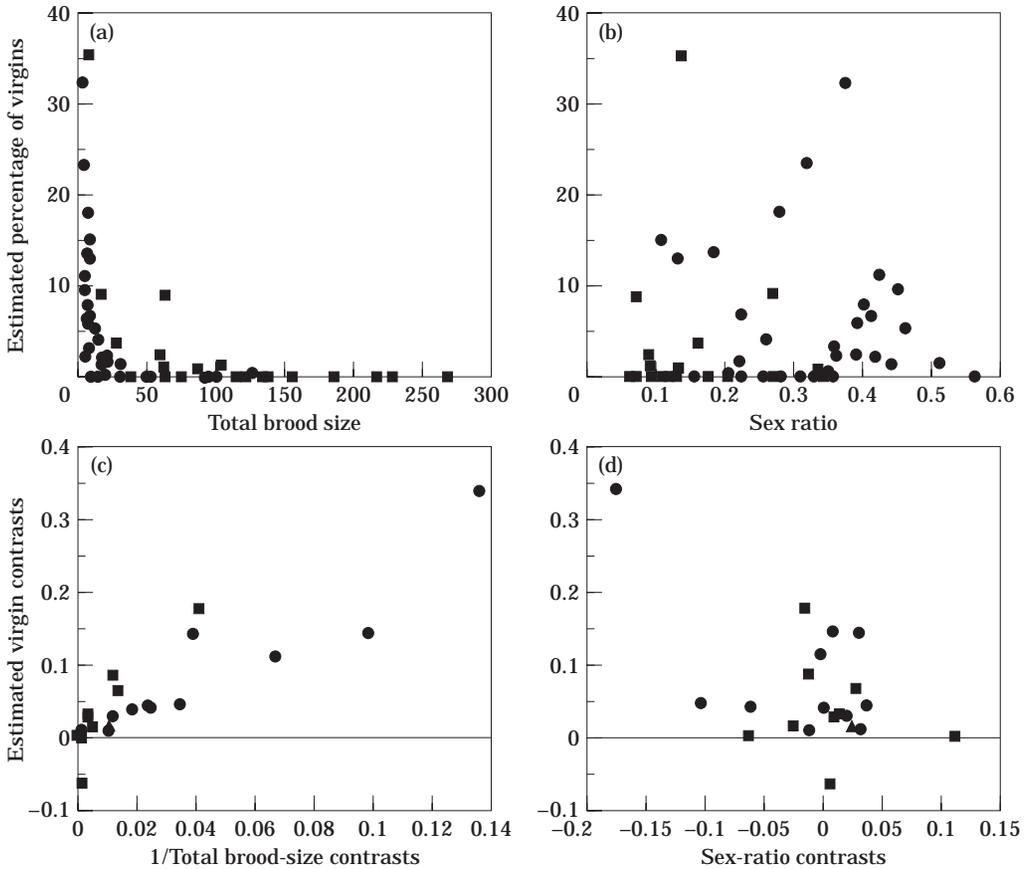
Figure 3. The relationship across fig wasp species between the estimated percentage of virgins (proportion of females developing in fruit with no conspecific males), the total brood size (average number of wasps, of that species, developing in a fruit), and the overall sex ratio. (a,b) Species as data points. ●: Non-pollinating species; ■: pollinator species. (c,d) The independent contrasts for the estimated percentage of virgins, 1/total brood size and sex ratio calculated using the Grafen (1989) method for calculating branch lengths. ●: Contrasts between non-pollinating species/taxa; ■: contrasts between pollinator species/taxa; ▲: the comparison between the pollinating and the non-pollinating species.

simpler single female case. Virginity should increase with (1) increasing male developmental mortality and (2) decreasing clutch size. The predictions further suggest that, under conditions of local mate competition, the average number of offspring developing in a patch is likely to be a far better predictor of virginity levels than the number of females that oviposit in a patch. Our model also predicts the coexistence of different sex-ratio strategies (e.g. laying either one or two males) under some circumstances. This result might contribute to an explanation for the existence of the much debated variation between female *Nasonia vitripennis* parasitoids in their

sex-allocation strategies, when ovipositing in small foundress groups (e.g. Orzack et al. 1991). Orzack & Sober (1994) have argued that such variation presents serious problems for the predictive power of optimality models in evolutionary biology (see also Godfray 1994; Herre 1995; Nagelkerke 1996; Herre et al., in press).

The estimates of the prevalence of virginity in fig wasps varied from 0 to 35.3% and represent the proportion of females that developed in fruit containing no conspecific (and wingless) males. Across species, there was no significant correlation between the estimated prevalence of virginity and our inferred index of local mate

competition, the overall sex ratio of a species. Consequently, the results do not support the prediction of Godfray & Hardy (1993) that the prevalence of virginity should be negatively correlated with the intensity of local mate competition. However, the estimated prevalence of virginity showed a significant negative correlation with the average number of offspring developing in a patch (Fig. 3), as predicted by both Heimpel (1994) and the models developed above. In addition, we argue below the small numbers of wasps developing in fruit are likely to reflect a high degree of local mate competition.

Three observations suggest that non-pollinating fig wasp species that have small average numbers of wasps developing in fruit (total brood size), and reasonable levels of virginity, are also subject to a high degree of local mate competition. First, if several conspecific females oviposited into each fruit, the total brood size would be larger (Werren 1983; Murray 1987; Greeff, in press). Second, the species with small total brood sizes generally also occur in a small proportion of fruit. Rare species that occur in a small proportion of fruit, and have exclusively wingless males, are likely to be subject to a high degree of local mate competition (S. A. West & E. A. Herre, unpublished data; see also Read et al. 1995). Third, the species with small total clutch sizes generally have sex-allocation patterns (high female bias and low between-brood variation) characteristic of extreme local mate competition (Green et al. 1982). For example, the Australian *Philotrypesis* sp. from *F. rubiginosa* has a mean total brood size of only 3.2, a sex ratio of 0.38 and less than binomial variance in brood sex ratio (J. M. Cook, unpublished data).

Will mated females be selected to adjust their offspring sex ratio in response to ovipositing virgin females? We have argued that the species with appreciable levels of virginity are likely to be subject to a high degree of local mate competition. Godfray (1990) has previously shown that mated females should not adjust the sex ratio of their offspring in response to the presence of virgins under conditions of extreme local mate competition. Consequently, high virginity levels appear to occur in situations where mated females should not adjust the sex ratio of their offspring in response to ovipositing virgins. If this pattern holds across the Hymenoptera then the presence of ovipositing virgin females is unlikely to have

played a major role in facilitating the evolution of eusociality.

How valid is the estimate of virginity that we have used in our study? The proportion of females developing in fruit that contain no conspecific males is likely to be strongly correlated with the prevalence of virginity (Godfray 1988). There are, however, some factors that might weaken the correlation. First, we would overestimate virginity levels if females were able to obtain mates after they left their natal fruit. We believe this to be unlikely as the wingless males are unable to survive outside the fruit (Cook et al. 1997). In addition, even if these males could survive outside the fruit they would be quickly preyed upon by ants, which are commonly observed, in numerous fig species, on the fruit from which wasps are emerging. Second, our method may underestimate the prevalence of virginity since it assumes that all females developing with conspecific males are mated (Godfray 1988). A study on five of the African fig wasp species considered here (S. A. West, S. G. Compton, S. L. Vincent, E. A. Herre & J. M. Cook, unpublished data) suggests that we are more likely to have underestimated virginity levels. West et al. compared the estimates of virginity levels obtained using our method with those based on the proportion of sticky-trap caught females without sperm in their spermathecae. Although the two methods gave very similar results and were correlated, the estimates obtained using our method were indeed slightly lower.

Whether a fig wasp species has winged, wingless or dimorphic (both winged and wingless) males correlates with pre-dispersal mating opportunities, which are determined by population density (Hamilton 1979; Greeff 1995; Cook et al. 1997; Herre et al., in press). At low population densities there is a high probability that a wasp may develop in a fruit with no members of the opposite sex. In this case, it would be advantageous to be a winged male, which can search for unmated females elsewhere. Conversely, at high population densities a male wasp will almost always develop in a fruit that contains several females. In this case, a male should concentrate on obtaining mates from his own fruit. Inside a fruit, wings are likely to impair freedom of movement, and the resources required to grow them could more beneficially be employed elsewhere (Hamilton 1979). At intermediate population densities, males have an intermediate probability of finding conspecific

females in their natal fruit. In this case a dimorphism with both winged and wingless males can be maintained if the fitness of the two morphs is equal (Hamilton 1979). Specifically, Hamilton (1979) suggested that the proportion of winged males in a species should equal the proportion of females that develop in fruit without conspecific wingless males.

Given the argument above, the existence of species with small average brood sizes, a high proportion of females developing in fruit without conspecific males, and only wingless males is surprising. A possible explanation for this is phylogenetic inertia. Wingless males are likely to evolve in species with a large mean brood size. However, if descendant populations and/or species undergo a substantial reduction in brood size, they may endure high virginity levels and strong selection for winged males, but lack the genetic variation for selection to act upon. Such a situation has been termed irreversible evolution (Bull & Charnov 1985; Godfray 1987; Harvey & Partridge 1987) and the genus *Philotrypesis* may provide one of many examples within the fig wasps. Some *Philotrypesis* species with wingless males have relatively large brood sizes (e.g. *P. pilosa* (26.2) and *P. spinipes* (25.3): Murray 1989) while others have very small brood sizes (e.g. *P.* spl (3.5): Murray 1989; *P.* sp *ex F. rubiginosa* (3.2): this study). If this scenario is correct, the proportion of winged males in a wasp species provides an example of a life-history trait that is obviously moulded strongly by selection, but may also be sub-optimal because of phylogenetic constraints.

APPENDIX

Suppose two female wasps oviposit on a patch. In what follows, we shall for simplicity assume diploid genetics. One female lays M eggs of which I are males; the other female also lays M of which J are males. The probability that a male egg suffers mortality is d and a female egg D; survival of young in a patch is independent. We assume that if a female develops in a patch in which at least one male survives she is mated and has a relative fitness of one. If she develops in a patch without males her fitness is reduced by a factor v which reflects the costs of finding a mate or of remaining a virgin. We treat v as a constant although we are aware that this is a simplification.

Consider the fitness of the I female. Her reproductive success equals the numbers of daughters she produces, taking into account their mating status, and the expected matings of her sons. We assume that her sons mate $i/(i+j)$ of the females in the patch where i is the number of her sons that survive and j is the number of the other female's sons that survive. Define $W(I,J)$ to be the fitness of the I female as a function of the number of males produced

$$W(I,J) = D\left\{ (M-I)(1-(1-v)d^{I+J}) \right.$$
$$+ (2M-I-J)\sum_{i=1}^{I}\sum_{j=0}^{I}\frac{i}{i+j}$$
$$\left. B(i;I,(1-d))B(j;J,(1-d)) \right\} \quad (A1)$$

where $B(i;I,(1-d))$ is the binomial probability of i successes out of I with parameter $(1-d)$. The first term in equation (A1) represents fitness through daughters, the second through sons.

The term in the double summation can be simplified using generating functions. Let $\Psi_i(s)$ and $\Psi_j(s)$ be the factorial moment generating functions of the probability distributions of i and j. Comins et al. (1980, equation B5) provided a theorem showing that the double sumation is given by

$$\int_0^1 \frac{d\psi_i(s)}{ds}\psi_j(s)ds. \quad (A2)$$

The generating functions are $\Psi_i(s) = ((1-d)s+d)^I$ and $\Psi_j(s) = ((1-d)s+d)^J$ and the required term is

$$\frac{I}{I+J}(1-d^{I+J}). \quad (A3)$$

Equation (A1) thus simplifies to

$$W(I,J) = D\left\{ v(M-I)d^{I+J} \right.$$
$$\left. + \frac{M(3I+J)-2I(I+J)}{I+J}(1-d^{I+J}) \right\}. (A4)$$

To obtain the optimum number of sons, we can proceed in two ways. The first is to treat I and J as continuous variables and to calculate $dW(I,J)/dI|_{I=J}=0$. I is then given by the implicit formula

$$\left(\frac{M}{2I} - 2\right)(1 - d^{2I}) - vd^{2I}$$

$$+ 2d^{2I}\ln(d)\left(1 - \frac{v}{2}\right)(I - M) = 0. \quad (A5)$$

Note that as the risks of male mortality (d) become small $J \rightarrow M/4$ which is the optimum sex ratio in Hamilton's local mate competition model for two foundresses. As d increases, equation (A5) predicts that more than a quarter of eggs should be male; although this effect is marked only when M is small, in which case the assumption that I and J are continuous is poorly met. Note, the optimum strategy is independent of female survival (D).

If I and J are treated as discrete, the evolutionarily stable strategy (ESS) can be described as a strategy P: produce broods containing I males with probability p_I. If $W(I,P)$ refers to the fitness of a female laying I eggs in a population playing the ESS, then the ESS is given by the conditions $W(I,P) = W(P,P)$ for $p_I > 0$; $W(I,P) < W(P,P)$ for $p_I = 0$. Note that D in equation (A4) will always cancel so that the ESS is independent of female developmental mortality.

In the case of a clutch size of $M = 4$ with the fitness of virgin females $(1 - v)$ assumed to be zero; if male mortality is low ($d < 0.42$), all females should produce one male, while if it is high ($d > 0.72$), they should all produce two males. For intermediate values, the ESS is to produce broods with a single male with probability $(6d^3 - d^2 - d - 1)/(3d^2(2d - 3))$.

Given the ESS male production strategy, P, the expected fraction of females leaving their natal patch unmated, V, is

$$V = \frac{\sum_{I=1}^{M} \sum_{J=1}^{M} p_I p_J d^{I+J}(2M - I - J)}{\sum_{I=1}^{M} \sum_{J=1}^{M} p_I p_J (2M - I - J)}.$$

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