

1 **Sexual selection on population-level mating opportunities drives morph ratios in a**
2 **fig wasp with extreme male dimorphism**

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15 **Abstract**

16 **Background**

17 Alternative mating tactics are widespread in animals and associated with extreme morphological
18 polymorphism in some insects. Some fig wasps have both highly modified wingless males and
19 dispersing winged males. Wingless males mate inside figs before females disperse, while winged
20 males mate elsewhere after dispersal. Hamilton proposed a model for this system with morphs
21 determined by alternative alleles. This has an equilibrium where the proportion of winged males
22 equals the proportion of females dispersing unmated; i.e. the proportion of matings that they
23 obtain. Previously, we have shown qualitative support for this prediction across nine fig wasp
24 species. Here I test the quantitative prediction in a population of the fig wasp *Pseudidarnes*
25 *minerva*. In addition, while Hamilton envisaged simple Mendelian strategies, some fig wasp
26 species with two wingless male morphs (but no winged males) show a conditional strategy with
27 morph determination influenced by the number of wasps developing in a patch - I also test for this
28 pattern in *P. minerva*.

29
30 **Results**

31 I sampled 114 figs that contained a mean of 2.1 *P. minerva* wasps from 44 trees across four sites in
32 Sydney, Australia. At the whole population level, the proportion of winged males (0.84 or 0.79
33 corrected for sampling bias) did not differ significantly from the proportion of unmated females
34 (0.84), providing strong quantitative support for the prediction of Hamilton's model. In addition,
35 there was no evidence for other factors, such as local mate competition or fighting between
36 wingless males, that could violate simplifying assumptions of the model. Meanwhile, the
37 proportion of winged males was not correlated with the number of wasps per fig, providing no
38 evidence for a conditional strategy.

39

40 **Conclusions**

41 Morph ratios in *P. minerva* are consistent with Hamilton's simple Mendelian strategy model,
42 where morph ratios are set by average mating opportunities at the population level. This contrasts
43 with some fig wasps from another subfamily that show conditional morph determination, allowing
44 finer scale adaptation to fig-level mating opportunities. However, these conditional cases do not
45 involve wing polymorphism. Male polymorphism is common and variable in fig wasps and has
46 evolved independently in multiple lineages with apparently different underlying mechanisms.

47

48 **Keywords:** mating systems, sexual selection, fig wasp, alternative strategies, male polymorphism

49

50

51 **Introduction**

52 Sexual selection is a pervasive force that can drive dramatic phenotypic diversity both between
53 and within the sexes (1, 2). Alternative mating tactics within a sex are known in many animal
54 species (2-5) and, in many cases, different behavioural tactics are matched by morphologies that
55 are also distinct (2, 3). The strategies underlying such polymorphism have been much debated
56 (e.g. (2-6)) and two general types of models have been proposed. The first involves a simple
57 genetic mechanism, involving alternative alleles at one or a few loci, where different phenotypes
58 arise from different genotypes (7, 8). This model requires morphs to have equal fitness to coexist,
59 since otherwise the fitter strategy would become fixed, and predicts that the equilibrium
60 proportion of each strategy equals the proportion of offspring that it produces (7-9). To date there
61 are relatively few good cases for species conforming to the alternative alleles model, but these
62 involve diverse taxa. For example, amongst vertebrates, genetic male morphs with different
63 mating behaviours occur in a bird (10), a lizard (11) and a fish (12). Among invertebrates a key
64 example is the marine isopod, *Paracerceis sculpta*, which has three male morphs determined by
65 alternative alleles (13) for which there is good evidence for equal average fitness (2, 14). Examples
66 from insects include the damselfly *Mnais costalis* (15) and most recently, a weta, *Hemideina*
67 *crassidens* (Orthoptera) (16). Most known examples involve male polymorphism, but female
68 genetic morphs occur in *Ischnura* damselflies and appear to be widespread in damselflies and
69 dragonflies (17).

70 In contrast to the above, there are numerous examples of the second form of morph
71 determination, where the phenotype expressed is a conditional strategy that depends on
72 environmental or social cues (2, 3, 5). For example, the mite *Rhizoglyphus echinopus* has a large
73 fighting and a small non-fighting male morph and their relative proportions are determined by
74 colony size (18). However, conditional strategies can also depend on genetic effects, since most

75 are thought to be threshold traits, where a key continuous variable (e.g. the level of a hormone or
76 juvenile growth rate) determines the morph produced (6, 19). Such threshold traits are generally
77 underpinned by many genes with small effect, so the threshold can evolve adaptively to match
78 local sexual selection pressures (6, 19, 20). For example, further studies of *R. echinopus*, using an
79 experimental evolution approach, have shown that the switch point between production of
80 sneaker and fighter male mites can be changed by manipulating habitat complexity (20).
81 Importantly, if conditional morph determination responds to local patch conditions, adaptation
82 can be more fine-grained than with a Mendelian strategy, where morph ratios may only be
83 optimized at the coarser population level (2, 6, 21). Consequently, some authors have argued that
84 Mendelian strategies should be rare since they will generally be outcompeted by a conditional
85 strategy with an evolving threshold (6). Together, the theoretical basis and empirical evidence for
86 the prevalence of conditional versus Mendelian strategies have formed a long-standing area of
87 ongoing debate.

88 Some of the most extreme cases of male polymorphism occur in fig wasps (8, 22-24), tiny insects
89 that develop inside the inflorescences (figs) of *Ficus* trees. Many non-pollinating fig wasps have
90 only winged males, which mate with females outside of figs (e.g. on fig leaves) after dispersal.
91 These species typically have very few individuals developing per fig (hereafter brood size). In
92 contrast, all fig-pollinating wasps have only highly modified wingless males, adapted to searching
93 for and mating with females inside the dark confines of the fig fruit (8, 22, 24, 25). These species
94 have large brood sizes (tens or hundreds of wasps developing per fig) and mating occurs inside the
95 fig before the females disperse. Many non-pollinating fig wasp species also conform to this
96 syndrome of large mating group size and wingless males (24). Between these extremes, lie some
97 species with intermediate brood sizes, in which winged and wingless male morphs coexist (see
98 Figure 1). In these wing-dimorphic species, wingless males mate with females inside figs, while

99 winged males exit figs and mate with unmated females after dispersal. Females either mate with
100 males inside their natal fig or leave the fig and disperse to mate with males elsewhere. Hamilton
101 (8) predicted that male dimorphism would persist only in species with intermediate (and/or highly
102 variable) brood sizes, which was supported by a subsequent comparative study (24).

103

104 While comparative analysis supports the general correlation between brood size and the existence
105 of winged and/or wingless males across species (8, 24), I am aware of no single species studies to
106 test quantitative predictions for morph ratios within wing-dimorphic species. Hamilton (8)
107 proposed a simple model for these male-haploid species, involving a single locus with alternative
108 alleles for winged and wingless males. Wingless male fitness derives from pre-dispersal mating
109 inside figs and winged male fitness from post-dispersal mating outside figs. Allele (and morph)
110 frequencies therefore depend on the relative frequency of pre- and post-dispersal mating
111 opportunities(8). An equilibrium should occur when the frequency of winged males equals the
112 frequency of females dispersing from their figs unmated. If wingless males are less common than
113 this, then they have more mating opportunities (higher fitness) and should increase in frequency.
114 If more common, they have fewer per capita mating opportunities (lower fitness) and should
115 decrease in frequency. Consequently, if we can measure the proportion of females dispersing
116 unmated, we can test if this equals the proportion of winged males. In fig wasps, the former can
117 be estimated by assuming that females developing in a fig with wingless males are mated by those
118 males, and then assessing how many females develop in figs that do not contain wingless males (8,
119 23). Meanwhile, the latter can be estimated from a large sample of males from the same
120 population.

121

122 To date, within-species studies of fig wasp male polymorphism have focused mainly on species
123 with more nuanced dimorphisms (see Figure 2) involving two (or more) types of wingless male (21,

124 26-30). Interestingly, there is good evidence for conditional morph determination, linked to brood
125 size, in two such wasp species from different genera (*Otitesella* and *Walkerella*) in the subfamily
126 Otitesellinae (21, 27, 30). Further, Pienaar & Greeff (21) pointed out that evidence across species
127 for frequency-dependent morph ratios does not discriminate between genetic or conditional
128 morph determination within species. Fig wasp life cycles can involve large fluctuations between
129 generations in the proportion of females dispersing unmated and genetic morph determination
130 would lead to a poor fit between morphs and mating opportunities in an individual fig or a crop of
131 figs on a tree. Pienaar & Greeff (21, 27) showed that this fit in *Otitesella pseudoserrata* was too
132 good to be consistent with alternative alleles, suggesting a conditional strategy. They further
133 argued that temporal fluctuations in mating opportunities make it unlikely that genetic morph
134 determination could persist in other fig wasps, but it seems premature to rule it out in general for
135 the following reasons.

136
137 First, male polymorphism has evolved independently in several fig wasp lineages (24, 31), leading
138 to hundreds of species with male polymorphism (23), and some may have genetically determined
139 morphs (23). Second, the cases discussed in the paragraph above do not involve wing dimorphism,
140 only the more subtle differences between wingless male morphs (Figure 2). Detection of different
141 wingless male morphs is sometimes easy, but in other cases can require detailed quantitative
142 analysis to reveal differences in the allometry of body parts (26, 29, 32). It is therefore easy to
143 imagine that the genetic and molecular mechanisms responsible for this type of variation (Figure
144 2) are different to those driving the striking major differences between winged and wingless males
145 (Figure 1). Third, beyond fig wasps, there are examples of genetically determined male morphs in
146 diverse vertebrate and invertebrate taxa (e.g. (10, 11, 13, 15, 16)).

147
148 In this study, I conduct the first detailed single species study of natural morph ratios and morph

149 determination in a fig wasp species that is wing-dimorphic - *Pseudidarnes minerva* (Figure 1)
150 associated with *Ficus rubiginosa* trees in Australia. This species was included as a single data point
151 in a previous comparative analysis of wing dimorphism across fig wasp species (24). The
152 proportion of winged males (0.77) was significantly higher than the estimated proportion of
153 females dispersing unmated (0.42), but the data were a by-product of a community ecology study
154 (33), with associated sampling limitations for the study of male dimorphism (see discussion). Here,
155 I use a new data set with more appropriate sampling to (1) estimate and compare the proportion
156 of winged males and females dispersing unmated at the population level; and (2) test if the
157 proportion of wingless males increases with the number of wasps in a fig, suggesting a conditional
158 strategy that allows fine-scale adaptation.

159

160 **Results**

161 **Population data**

162 *P. minerva* wasps only emerged from about 1 in 25 figs collected, resulting in 243 wasps from 114
163 figs from 44 trees at the four sites across Greater Sydney (Table 1). There was a mean of 2.13
164 wasps per fig and most (85%) occupied figs contained only 1-3 wasps (Figure 3), suggesting that it
165 is rare for females to lay more than 1 or 2 eggs per fig. This also provides very little opportunity for
166 local mate competition. The overall population sex ratio (proportion males = 0.33) was female-
167 biased ($\chi^2 = 27.671$, $df = 1$, $p = 1.438e-07$), as in most other non-pollinating fig wasp species with
168 local or partial local mating (West et al. 1998; Fellowes et al. 1999).

169

170 **Testing for equal fitness of male morphs**

171 At the population level, the proportion of winged males was 0.838 (67/80), which is not
172 significantly different ($\chi^2 < 0.001$, $df = 1$, $p = 1$) from the estimated proportion (137/163 = 0.841) of
173 females dispersing unmated, supporting the key quantitative prediction. In addition, pWM and

174 pUF were not significantly different at any of the four sites when tested individually (Table 1) and
175 neither pWM ($\chi^2 < 1.47$, $df = 3$, $p = 0.69$) nor pUF ($\chi^2 < 4.76$, $df = 3$, $p = 0.19$) differed significantly
176 between sites.

177
178 However, my sampling may have underestimated the proportion of wingless males, due to the fact
179 that, unlike winged males and females, they do not usually emerge from figs. While individual
180 females, winged or wingless males may fail to develop fully or emerge successfully from their galls,
181 these “failed” wasps are not expected to be biased towards a particular gender or morph. In
182 contrast, amongst wasps that “succeed”, i.e. emerge from their galls and carry out normal
183 subsequent behavior, there is a bias in my method against detecting wingless males. Figs were only
184 dissected to reveal wasps remaining inside them if at least one wasp had emerged from the fig, and I
185 had already excluded any figs from which a winged male or female may have already dispersed
186 before collection (see methods). Since winged wasps emerge from figs to disperse, my counts of
187 winged wasps should be accurate. However, most wingless males remain inside figs and so the data
188 set may be missing rare figs containing only wingless male(s). This number of “missing wasps” can be
189 estimated using the population data for mean numbers of wasps per fig, sex ratio and male morph
190 ratio (Table 1; Supplementary Information File 1).

191
192 We can calculate the expected number of cases where there would be just one male (a singleton),
193 either winged or wingless, by dividing the observed number (16) of singleton winged males by the
194 observed proportion ($51/64 = 0.80$) of winged males in figs with two or more wasps. The justification
195 for this is that we should have accurate estimates of both the number of singleton winged males,
196 and the male morph ratio in samples where at least one winged wasp (male or female) was present.
197 This yields an expected figure of 20.08 singleton males, implying 4.08 missed singleton wingless
198 males. If we now consider the 21 figs with two wasps, only four have two males. Three cases have

199 two winged males and one has a winged and a wingless male. The probabilities of different male
200 combinations are: two winged (0.635), one of each (0.324), and two wingless males (0.041). It is
201 therefore highly unlikely that any cases of two wingless males were missed, but we can include a
202 correction factor of $0.041 \times 4 \times 2 = 0.38$, to yield a total of 4.46 missing wingless males. By extension, a
203 group consisting of only three wingless males is even more unlikely, and there are no cases of three
204 or more males (of any type) at all in the empirical data, so I ignore this trivial possibility. If we now
205 calculate the corrected proportion of winged males $67/84.46 = 0.793$, this remains not significantly
206 different ($\chi^2 = 0.57257$, $df = 1$, $p = 0.4492$) from the proportion of females dispersing unmated
207 (0.841).

208

209 **Testing for conditional morph and sex ratios**

210 Neither the likelihood of being a winged male ($z = -0.27$; $P = 0.79$; $d.f. = 1, 58$) nor the sex ratio ($z =$
211 -1.24 ; $P = 0.22$; $d.f. = 1, 12$) changed significantly as the number of wasps in a fig increased (Figs
212 4,5). Hence, there is no evidence that either of these binomial variables are adjusted adaptively
213 according to fig contents.

214

215 **Opportunity for local mate competition**

216 A total of 13 wingless males was recorded. In 11 figs there was only one wingless male, while one
217 fig contained two wingless males. Consequently, there is very little opportunity for local mate
218 competition between wingless males in the same fig.

219

220 **Discussion**

221 This is the first detailed single species study of natural morph ratios in a fig wasp showing extreme
222 male dimorphism (Figure 1). Overall, the results are consistent with Hamilton's (8) model that
223 proposed morph ratios determined by frequency-dependent selection on their respective mating

224 opportunities. I found that the proportion of *P. minerva* winged males (0.84) was not significantly
225 different from the proportion of females dispersing unmated (0.84 or 0.79 corrected for sampling
226 bias). This supports the hypothesis that sexual selection on mating opportunities defines the
227 equilibrium proportion of winged males at the population level. In addition, the proportion of
228 winged males is not correlated with the number of wasps in a fig (brood size). This is consistent
229 with simple Mendelian segregation of alleles, but not with morph determination that is
230 conditional on mating opportunities in the local patch (fig), as seen in some other fig wasps, where
231 the proportion of wingless males increases with the number of wasps in a fig (23, 27). Hence male
232 morph proportions in *P. minerva* appear to be set by their average mating success at the
233 population level, but not adjusted more finely to patch level opportunities.

234

235 The *P. minerva* population level data are consistent with Hamilton's simple model of morph
236 proportions (8). However, various details of male behavior could decrease the reproductive value
237 of wingless males relative to winged males and therefore select for an increased proportion of
238 winged males. First, LMC between wingless males in the same fig could reduce their reproductive
239 value and select for a higher proportion of winged males (34, 35). However, in this study the
240 average number of *P. minerva* wasps per fig was very low (2.13; Table 1) and only one fig
241 contained more than a single wingless male, so LMC can be no more than a trivial force. Second,
242 some females may disperse unmated from patches with wingless males, especially if there is lethal
243 fighting between males (24, 35). Fighting occurs between wingless males of some wing-dimorphic
244 species (8), but not *P. minerva* whose wingless males (Figure 1) lack the large jaws and armour of
245 fighting fig wasps (25). Moreover, they also lack the opportunity to fight as they so rarely co-occur
246 with other wingless males. Third, winged males might sometimes mate within their natal fig with
247 females (24, 35, 36), usurping some mating opportunities assumed to be taken by wingless males.
248 This is harder to dismiss, but I found no evidence for it in the current study, where all winged

249 males either emerged from figs or (only one) were unhatched from their galls. In contrast,
250 wingless males have been observed to bite a small hole into galls containing females and crawl
251 inside them to mate with the female inside the gall (24). Consequently, the known biology of *P.*
252 *minerva* does not suggest that any of these factors are significant.

253

254 I found no evidence that morph determination was conditional upon the number of wasps
255 developing per fig. This contrasts with studies of *Otitesella* wasps (21, 27) that have no winged
256 males but two wingless morphs (Figure 2), where the proportion of the disperser morph decreases
257 as the number of wasps in a fig increases. As the number of females in a fig increases, so do the
258 mating opportunities for resident rather than dispersing males, and *Otitesella* wasps show fine-
259 grained adaptation of morph ratios to local patch conditions (21, 27). Such conditional morph
260 determination allows more precise adaptation to mating conditions in the local patch and is also
261 shown in *Rhizoglyphus echinopus* mites, which have morph proportions determined by colony size
262 (18). Such precise adaptation is not shown by *P. minerva* and is not possible under simple genetic
263 control. Consequently, there seem to be at least two different mechanisms underlying male
264 dimorphism in fig wasps. Male dimorphism is extreme in *P. minerva* (37), with major differences in
265 morph size, appendages, and pigmentation, as well as the most obvious difference of wings
266 (Figure 1). In contrast, the differences between the wingless *digitata* and *religiosa* morphs of
267 *Otitesella* wasps (Figure 2), while still striking, are more modest. In particular, as both morphs lack
268 wings, even the dispersers are unlikely to travel beyond one or more branches on the same tree,
269 so local (tree level) conditions are relevant to the dispersers as well as the non-dispersers. It is
270 therefore not so surprising that a different mode of morph determination operates in these two
271 cases, even though they have several aspects of selection in common.

272

273 An interesting case that falls between the *Pseudidarnes* and *Otitesella* examples of male

274 polymorphism involves a fig wasp from a third different subfamily of wasps (Epichrysomallinae).
275 *Sycobia* sp. has both winged and wingless males, but apart from the lack of wings and slightly
276 smaller eyes in the wingless morph, the two morphs are similar in size and general appearance
277 (38). Niu et al. (38) found that the proportion of wingless males decreased with brood size,
278 suggesting a conditional strategy. However, they confined females in small bags with figs and
279 obtained brood sizes of up to 400 wasps per fig with a mean of over 100. It seems likely that the
280 changes in morph ratio with brood size occurred in very large broods that would not occur under
281 natural conditions. No data were presented on natural morph ratios or brood sizes, but for
282 context the species mean brood size for the nine male-dimorphic fig wasp species in a
283 comparative analysis was just seven (Cook et al. 1997).

284

285 Although my analyses here show that *P. minerva* morph ratios are not conditional on brood size, I
286 did not test directly for genetic morph determination - an obvious next step. The male morphs
287 produced by individual *P. minerva* females could be compared with the predictions of a single
288 locus model. With the winged (+) allele at a frequency of 0.8 and the wingless (-) allele at 0.2 there
289 should be three female genotypes with frequencies of 0.64 (++) , 0.32 (+-) and 0.04 (--). In
290 principle, a manipulative experiment could be used to produce arrays of offspring from females to
291 compare with these expectations (38). However, the logistics are challenging. First, the intricate
292 life cycle requires females to be given access to figs at the right stage of development that have
293 not been exposed to other conspecific wasps. Second, the figs must then be kept free from other
294 conspecifics wasps that might lay eggs, but able to ripen normally on the tree. Third, females seem
295 to lay only one or two eggs per fig (Table 1), so many figs would probably be needed per female to
296 get suitable offspring numbers, and there is a risk of females laying unusually high numbers of
297 eggs per fig (38), as discussed above. An alternative would be to use molecular markers, such as
298 microsatellites, to reveal parentage of unmanipulated wasps. This approach has been used

299 successfully on other non-pollinating fig wasps to reveal the number of females laying eggs per fig
300 and the number of offspring laid in a fig per mother (39, 40). However, it rarely recovered
301 offspring from the same female in different unmanipulated figs, presumably due to the dilution
302 effect of large numbers of figs per tree and the option for females to visit many trees (39, 40).
303 Perhaps the best approach would be to combine manipulations and microsatellites to try to obtain
304 offspring arrays from some females confined in bags around figs, then screen all emerging males
305 genetically to identify brothers. An alternative approach is to use whole-genome sequencing of
306 winged and wingless males and bioinformatics to search for consistent genetic differences
307 between them (41). However, while this may be good at revealing various genes that are
308 differentially expressed and involved in traits such as eye and wing formation (38), these are
309 unlikely to be the ones involved in a primary morph-determining signal.

310

311 Previous analyses of natural morph proportions in wing-dimorphic fig wasps (8, 24) have suffered
312 from low sample size per species and/or data that are the by-product of other studies with
313 different aims. In the current study, I sampled with the aim of estimating population averages for
314 the two key variables (proportions of winged males and unmated females) for *P. minerva*. Hence
315 the data derive from a small number of figs from each of a large number of trees, whereas most
316 previous studies sampled data from many figs from only a few trees. Similarly, my samples were
317 accumulated over many sampling sessions across four sites and two years, whereas many studies
318 involve only one site and one or two sampling periods. The sampling of the current study is
319 therefore better suited to estimating population averages for fig wasps whose abundance is highly
320 variable in space and time (8, 27). However, I also incorporated a shortcut that introduces
321 sampling bias – only figs from which at least one wasp exited were dissected to reveal any further
322 wasps within. Since wingless males rarely exit figs, this method could miss some figs that contain
323 only a wingless male, or a rare winged wasp that failed to exit successfully. I used the population

324 data to infer the existence of four such “missing wingless males”, which is conservative since I
325 ignored the possibility of missing winged males (or females). Applying this correction decreases
326 the proportion of winged males from 0.84 to 0.79, but this remains not significantly different to
327 the proportion of unmated females (0.84). Given that only about one in 25 figs contain *P. minerva*
328 and dissection of one fig takes about two hours, it would have required an extra 5000 hours of
329 microscopy to dissect all figs sampled. Interestingly, we obtained a similar estimate of the
330 proportion of winged males (0.77) using data collected as part of a fig wasp community ecology
331 study in Melbourne some 25 years ago (33).

332
333 In contrast, the estimated proportion of females dispersing unmated was much lower in the
334 earlier study (0.42) than observed here (0.83). Hence the frequencies of unmated females and
335 winged males were significantly different and provide further evidence for lack of conditional
336 adjustment of morph ratios to local mating opportunities. The earlier study involved figs from only
337 four crops of fruit at a single site and was conducted outside the natural range of the host plant
338 and its associated wasps. Surprisingly, a far higher proportion ($95/145 = 0.66$) of figs were
339 occupied by *P. minerva*, which could indicate that the species is much more abundant in
340 Melbourne, perhaps due to fewer competitor species outside its native range. Interestingly, high
341 *P. minerva* abundance has also been reported for an introduced population in New Zealand (42).
342 However, several common fig wasp species from Sydney are also found in Melbourne (33), where
343 the host plant is now well-established and common. I suspect that in studying only four fruit crops
344 at one site we happened to sample crops where *P. minerva* was unusually abundant. In this
345 context Hamilton (8) hypothesized that male wing-dimorphism might also be associated with high
346 variability in wasp abundance, i.e. high variation in mating opportunities for winged and wingless
347 males, and Pienaar & Greeff (21) also noted that such variation is common in fig wasps. The genus
348 *Pseudidarnes* was recently revised by Farache et al. (37), but has received little ecological or

349 behavioural study. The only population data for congeneric species are for *P. cooki*, which is
350 associated with another Australian fig species, *F. obliqua*. Segar and Cook (43) analyzed data from
351 11 different sites and found it occurred in only 1 in 20 figs with rarely more than four wasps in the
352 same fig. These population metrics are very similar to those reported for *P. minerva* in the current
353 study (Table 1) and suggest that the high abundances of *P. minerva* reported outside its natural
354 range may be atypical. To date, wingless males are not known for *P. cooki*, but it seems likely that
355 they may exist.

356

357 4.6. Conclusions and future work

358 In conclusion, this first detailed study of a fig wasp species with male wing-dimorphism supports
359 the hypothesis that the frequencies of winged and wingless males are maintained by sexual
360 selection on their mating opportunities. As the density of wasps increases, there are more mating
361 opportunities for wingless males inside figs and fewer females disperse unmated. Those females
362 emerging unmated (from figs without wingless males) are able to mate with winged males after
363 dispersal. Consequently, selection should adjust the frequency of the winged morph to equal the
364 proportion of females dispersing unmated, and we observe a very close correspondence of these
365 two values in *P. minerva*. However, there is no correlation between morph proportions and the
366 number of wasps per fig, suggesting absence of the conditional morph determination seen in
367 some *Otitesella* fig wasps that have resident and disperser wingless male morphs (21, 27). This
368 means that morph proportions in *P. minerva* are adjusted to average mating success at the
369 population level, but not at the patch (fig) level. Such coarse-grained adaptive fit is consistent with
370 a simple Mendelian strategy, which now requires more direct genetic testing. Both wing-
371 dimorphism and polymorphism amongst wingless males are common in fig wasps (8, 23, 24, 31)
372 and great diversity can be found even within and across species from the one genus *Philotrypesis*,
373 revealing considerable evolutionary lability (31). Studies of other genera and species would be

374 very interesting to probe whether conditional morph determination is linked particularly to cases
375 with multiple wingless morphs, and genetic control to cases with wing-dimorphism.

376

377 **Methods**

378 **Sampling**

379 In monoecious fig species, individual trees flower asynchronously and sporadically, such that there
380 is year-round flower and fruit production at the population level. From 2013 to 2015, monthly
381 sampling was conducted from *F. rubiginosa* trees at four sites in Greater Sydney - Manly, Balmoral,
382 Penrith, Wisemans Ferry. The target sample size was >50 figs pooled from >4 trees per site, but
383 due to the phenology described above, there were sampling sessions when either the number of
384 trees in fruit or the number of ripe fruits was lower.

385

386 Figs were collected when wasps were about to emerge from the fruits; i.e. when figs are yellow
387 and slightly soft. The wasp species fall into two distinct size categories - large and small (44). Large
388 wasps, including *P. minerva*, can only exit figs through holes that they chew themselves, not
389 through the very small holes made by the numerous pollinator wasps. It is therefore easy to tell
390 whether any large wasps, such as *P. minerva*, have already emerged. Based on this, we excluded
391 any figs that already had large holes when they were collected, since *P. minerva* wasps may have
392 already dispersed from these samples. The remaining individual figs were placed in 70 ml plastic
393 pots with gauze-covered tops (to prevent excess humidity and fungal growth) to allow recording
394 of all wasps emerging. After allowing 48 hours for wasps to emerge, figs from which at least one *P.*
395 *minerva* had emerged were dissected to find and count any further *P. minerva* wasps remaining
396 inside. This method provided counts of all *P. minerva* wasps in many figs, while not wasting large
397 amounts of time (about 2 hours per fig) dissecting the vast majority of figs (>95%) that do not
398 contain *P. minerva* wasps. It is, however, biased against detection of any figs that might contain

399 only wingless males, which I address below (see results and discussion).

400

401 **Population proportions**

402 For each fig, I recorded the number of *P. minerva* females, winged and wingless males and used
403 these data to generate population totals and means for various metrics. In particular I calculated
404 the overall proportion of winged males (pWM) and proportion of females dispersing unmated
405 (pUF), i.e. those developing in a fig with no wingless males ([8](#)). I then tested the morph ratio
406 prediction of Hamilton's model by comparing these proportions using a 2-sample test for equality
407 of proportions (prop.test function in R). In addition, I made the same 2-sample comparison for
408 each of the four sites. I then conducted separate 4-sample tests for equality of proportions to
409 compare pWM and pUF across sites. Finally, I also calculated the overall population sex ratio
410 (proportion males / all wasps) and tested if this differed from equality (0.5) again using the
411 prop.test function.

412

413 **Testing for conditional morph and sex ratios**

414 I then used the data to test if pWM decreases as the number of conspecific females in a fig
415 increases, as predicted under conditional morph determination ([23](#)) and reported for some fig
416 wasp species ([27](#), [30](#), [38](#)). This used a generalised linear model (GLM) with binomial errors in R. In
417 addition, I also conducted a similar analysis to test if the sex ratio increased with the number of
418 wasps in a fig.

419

420 **Opportunity for local mate competition**

421 Hamilton's model assumes that females lay one egg per patch and is effectively a special case of
422 Greeff's ([34](#)) more complex model, which allows females to lay multiple eggs per fig and therefore
423 creates the opportunity for local mate competition (LMC) between wingless males. The simpler

424 model is justified if empirical data show that LMC occurs only rarely in natural situations (e.g. see
425 Pienaar & Greeff (27)). Consequently, I calculated the frequency of figs with two or more wingless
426 males, i.e. those where LMC could occur.

427

428 **Supplementary information**

429 Supplementary information is provided in Additional File 1

430

431 **Declarations**

432 **Ethics approval and consent to participate** – not applicable

433 **Consent for publication** – not applicable

434 **Competing interests** – The authors declare that they have no competing interests.

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436

437 **Authors' contributions** – JMC: study concept and design; sampling and data collection; data
438 analysis; manuscript drafting and revision

439

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446

447 **Availability of data and materials**

448 All data generated or analysed during this study are included in this published article including

449 Supplementary information in Additional File 1.

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551

Tables and Figures

Table 1. Site and population level sampling of *P. minerva* in Sydney.

Site	figs	trees	FU/F	+M/M	chisq	p	wasps	wasps/fig	SR
Balmoral	42	17	48/55	24/27	<0.001	1	82	1.95	0.33
Manly	21	11	28/31	12/16	0.93	0.33	47	2.14	0.34
Penrith	22	8	37/50	14/17	0.14	0.71	67	2.91	0.25
Wisemans	27	8	22/27	17/20	<0.001	1	47	1.74	0.43
Sydney (all)	114	44	135/163	67/80	<0.001	1	243	2.13	0.33

FM = females mated; FU/F = females unmated/total females; +M/M = winged males/all males; chisq = chi-squared value for 2-sample test for equality of proportions; p = p-value; wasps = total wasps; wasps/fig = mean wasps per fig (see Fig. 3 for variation); SR = sex ratio (proportion males/all wasps).

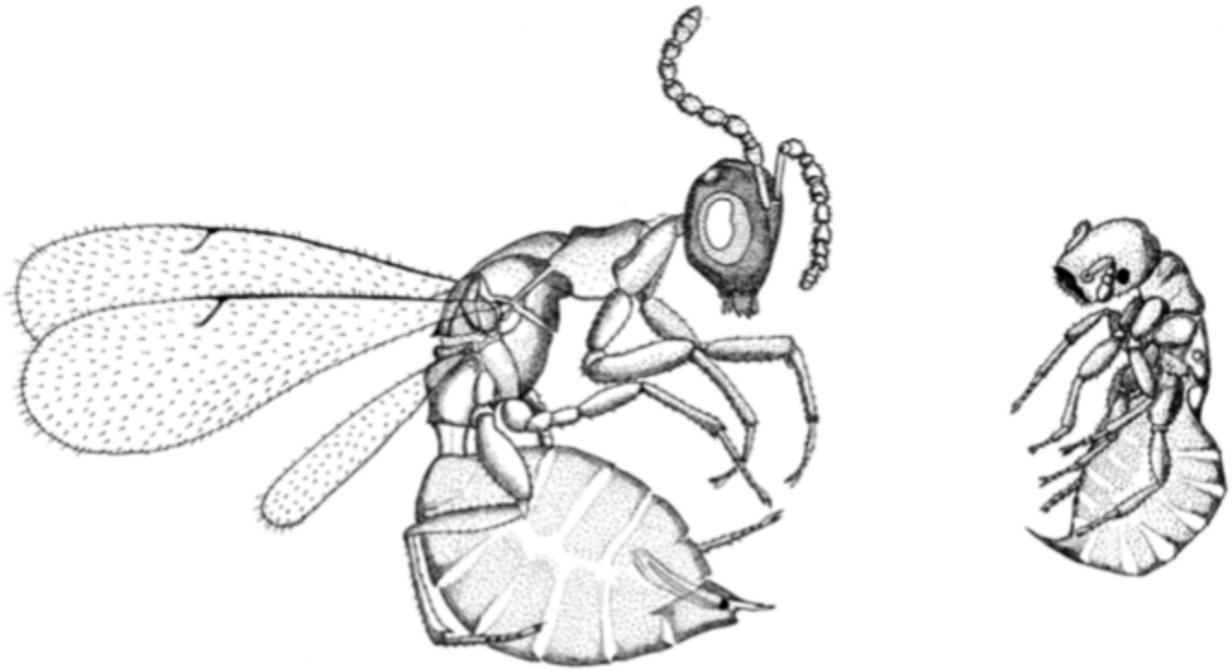


Figure 1. The winged and wingless male morphs of the fig wasp *Pseudidarnes minerva* provide an example of extreme male dimorphism. Original figure from Cook et al. (22) was drawn by Joanne Martin.

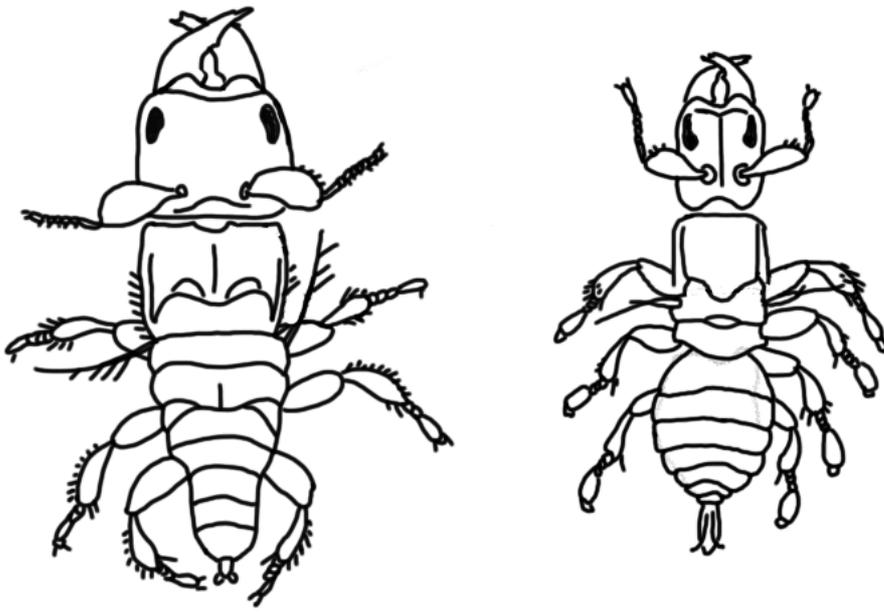


Figure 2. Less extreme wingless male dimorphism in *Otitesella* species. The larger, more combative *digitata* morph (left) and the smaller, more dispersive, *religiosa* morph (right). Drawings modified by JMC after Westwood 1883.

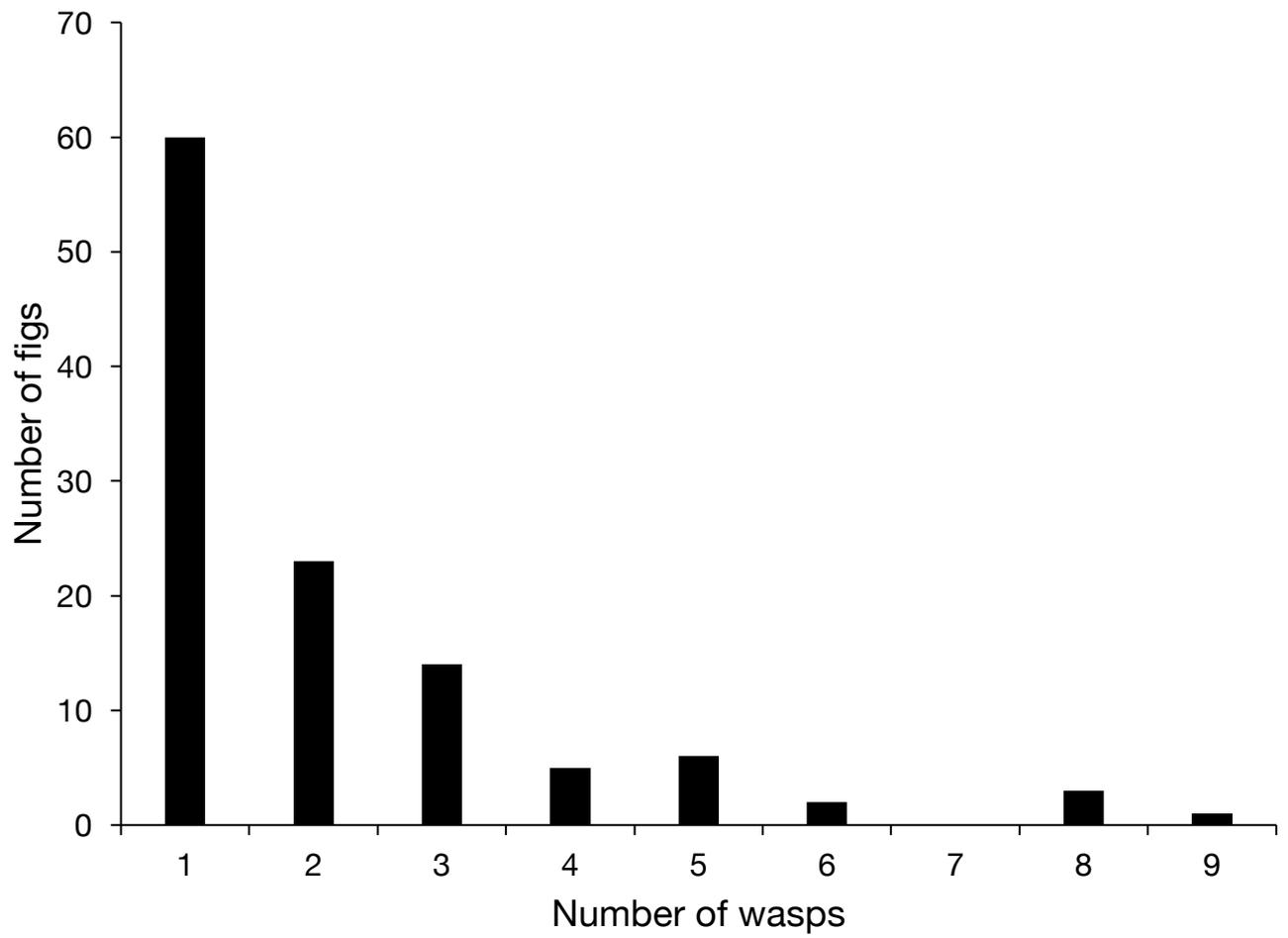


Figure 3. Numbers of *P. minerva* wasps in 114 occupied figs.

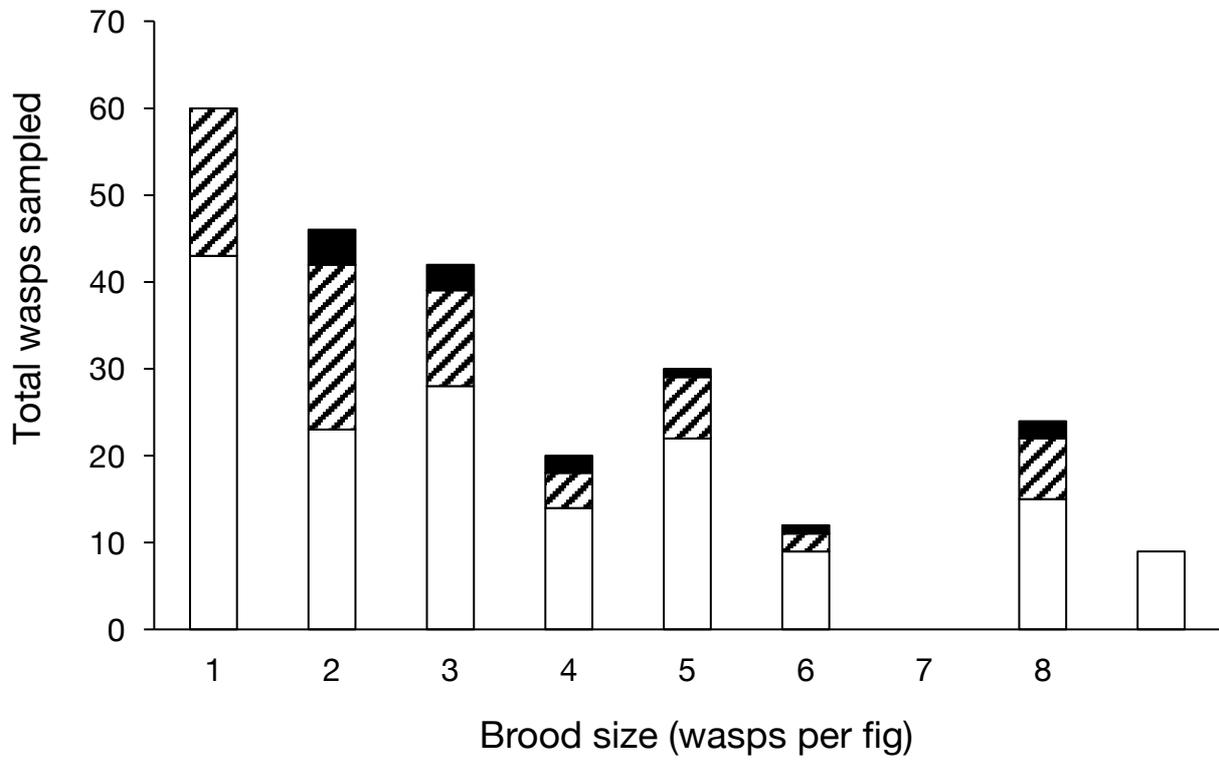


Figure 4. Numbers of females (white bars), winged males (hatched) and wingless males (black) in 114 figs with different numbers of wasps. NB singleton wingless males are not detectable with our sampling method - see text for further details.