

For: Entomologia Experimentalis et Applicata

Mark Jervis Memorial Special Issue

**Sex ratios, virginity and local resource enhancement in a quasisocial parasitoid**

Apostolos Kapranas<sup>1\*</sup>, Ian C.W. Hardy<sup>2</sup>, Xiuyun Tang<sup>3</sup>, Andy Gardner<sup>4</sup> & Baoping Li<sup>3</sup>

<sup>1</sup>Department of Biology, National University of Ireland Maynooth, County Kildare, Ireland

<sup>2</sup>School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK

<sup>3</sup>School of Plant Protection, Nanjing Agricultural University, No. 1 Weigang, Nanjing, Jiangsu 210095, P.R. China

<sup>4</sup>School of Biology, University of St Andrews, Sir Harold Mitchell Building, Greenside Place, St Andrews, KY16 9TH, UK

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\*Correspondence author:

Dr Apostolos Kapranas, Senior Postdoctoral Scientist, Behavioural Ecology and Biocontrol, Laboratory, Department of Biology, National University of Ireland, Maynooth, Co Kildare, Ireland.

tel: (+353) 1 708 6852

akapranas@gmail.com

Apostolos.Kapranas@nuim.ie

28 **Abstract**

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30 *Sclerodermus harmandi* is an economically beneficial species of parasitoid wasp that also has  
31 an unusual level of sociality: groups of female foundresses reproduce on a single host and  
32 exhibit cooperative post-ovipositional brood care. The beneficial effects females have on  
33 each other's reproductive success provides, via the theory of local resource enhancement  
34 (LRE), an explanation for their female biased progeny sex ratios which is part of the same  
35 framework for understanding sex ratio evolution as the more often invoked theory of local  
36 mate competition (LMC). Here we show that *S. harmandi* sex ratios are over-dispersed, with  
37 high variance largely attributable to the common occurrence (60%) of developmental  
38 mortality. Developmental mortality is also **positively** associated with the proportion of broods  
39 which contain only females at emergence (virgin broods). Virginity is also more common  
40 when broods are produced by smaller numbers of foundresses. Virginity is expected to be  
41 disadvantageous under LRE, as it is under LMC, but theory for LRE is less extensively  
42 developed. We suggest approaches for the development of LRE theory, in particular using  
43 models of "population elasticity" in which the intensity of kin competition is reduced because  
44 extra resources are available to local populations that are more cooperative. For *S. harmandi*,  
45 such extra resources may include large hosts that can only be successfully utilized when  
46 multiple foundresses cooperate.

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50 **Key words:** *Sclerodermus harmandi*, Bethyridae, LRE, LMC, developmental mortality, all-  
51 female broods

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## 53 Introduction

54 Sex ratios in many species of gregarious and quasi-gregarious parasitoid wasps are female  
55 biased. In the vast majority of cases this can be explained by the theory of local mate  
56 competition (LMC) (Hamilton, 1967, 1979; Godfray, 1994; West, 2009). LMC theory  
57 predicts that female biased sex ratios are selected for when the offspring produced by one or a  
58 few mothers, termed foundresses, mate among themselves before the female offspring  
59 disperse away from the natal group, a common feature of many parasitoid species (Hamilton  
60 1967, 1979; Godfray, 1994; Hardy, 1994; Godfray & Cook, 1997). LMC theory further  
61 predicts that bias will be less extreme when more foundresses contribute offspring to the  
62 mating group and such facultative adjustment of sex ratios is observed in empirical and  
63 comparative studies of parasitoids (Charnov, 1982; Godfray, 1994; Hardy, 2002; Hardy et al.,  
64 2005; West, 2009). In short, LMC theory has been a major key to the understanding of  
65 parasitoid sex allocation (Charnov 1982; Godfray, 1994; West, 2009; a critique of this  
66 success is provided by Orzack, 2002).

67 There are, however, some species of parasitoid wasps in which observed sex ratios are  
68 more biased than predicted by current LMC theory and/or do not vary with foundress  
69 numbers according to LMC predictions (Shuker et al., 2004, 2005; Matthews et al., 2009;  
70 Innocent et al., 2010; Abe et al., 2014; Tang et al., 2014). For instance, Abe et al. (2014)  
71 recently highlighted that the extremely female biased (1-5% males), and relatively invariant,  
72 sex ratios in the genus *Melittobia* (Hymenoptera: Eulophidae) are inexplicable using current  
73 sex ratio theory. A concurrent publication by Tang et al. (2014) on parasitoids in the genus  
74 *Sclerodermus* (Hymenoptera: Bethyilidae) similarly reported highly female biased sex ratios  
75 and, while sex ratios increased significantly with foundress number, the effect was not strong.  
76 Like Abe et al. (2014), Tang et al. (2014) concluded that the observed sex ratios could not be  
77 explained by LMC but in contrast to Abe et al. (2014) it was concluded that current sex ratio  
78 theory does provide an explanation: *Sclerodermus harmandi* is a quasisocial parasitoid  
79 (groups of females reproduce on a single host and exhibit cooperative brood care) and the  
80 beneficial effects females have on each other's reproductive success is expected to lead to  
81 female bias due to local resource enhancement (LRE), **wherein an excess production of the  
82 sex that leads to a greater increase in fitness of the parents or their offspring is favoured**  
83 (Taylor, 1981; West, 2009). **This was the first report of LRE operating among parasitoid  
84 wasps and one of only a small number of reports on LRE within the Hymenoptera (Schwarz,  
85 1988; Martins et al., 1999; Harradine et al., 2012). Although it is part of the same conceptual**

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3 86 framework for understanding the evolution of sex allocation strategies, theory for LRE has  
4 87 not been as extensively developed as LMC theory (Taylor, 1981; West, 2009; Gardner &  
5 88 Ross, 2013). In consequence, expectations for sex ratio means and variance and patterns of  
6 89 brood sexual composition in relation to other life-history variables such as foundress number,  
7 90 clutch size or developmental mortality, are less well defined than under LMC (e.g., Green et  
8 91 al., 1982; Werren, 1983; Griffiths & Godfray, 1988; Heimpel, 1994; Hardy et al., 1998).

92 In this companion paper to Tang et al. (2014) we summarize the pertinent biological  
93 details of *Sclerodermus harmandi* and then, using the original data set of Tang et al. (2014),  
94 we further explore how its sex ratios are affected by foundress number and group size and  
95 also how developmental mortality and group size influence the incidence of virgin (all-  
96 female) broods. We go on to suggest how theory for LRE and quasisociality might be  
97 developed by consideration of the biology of *Sclerodermus*.

#### 98 99 **Biology of *Sclerodermus harmandi***

100 *Sclerodermus harmandi* (Hymenoptera: Bethyilidae) is a gregarious ectoparasitoid of wood  
101 boring Cerambycid beetle larvae and is used extensively in biological control of coleopteran  
102 forest pests in China (Chen & Cheng, 2000). These beetles damage trees directly by feeding  
103 on them and also vector the extremely damaging pine wood nematode *Bursaphelenchus*  
104 *xylophilus* that causes pine wilt disease (Yang et al., 2014).

105 In marked contrast to the aggressive behaviour observed when adult females in some  
106 other genera of the Bethyilidae compete for exclusive access to a host (Hardy et al., 2013),  
107 *Sclerodermus* females appear to engage cooperatively in host suppression, oviposition and  
108 offspring care (Bridwell, 1920; Wheeler, 1928; Kühne & Becker, 1974; Mamaev, 1979;  
109 Casale, 1991; Hu et al., 2012; Wu et al., 2013), often producing very large broods of  
110 offspring (>100). Using *S. harmandi*, Tang et al. (2014) demonstrated experimentally that  
111 individual females increase their reproductive success by jointly exploiting large hosts, thus  
112 identifying the selective advantage of their quasisocial behaviour.

113 Some *Sclerodermus* species exhibit extremely female biased sex ratios (Griffiths &  
114 Godfray, 1988; Hardy & Mayhew, 1998; Li & Sun, 2011; Hu et al., 2012; Tang et al., 2014).  
115 While most offspring groups consist mainly of females, some consist entirely of females  
116 ('virgin broods'). In *S. harmandi*, when males are present they are relatively short lived and  
117 typically mate with maturing brood-mate females when these emerge or prior to their  
118 emergence by chewing entrances into their cocoons (Zhang & Tian, 1985; Hu et al., 2012).

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## 120 **Materials and Methods**

121 *Sclerodermus harmandi* was cultured at the Forestry Institute of Jiangsu Province, PR China  
122 where it is mass-produced for **release** as an agent of biological control of *Monochamus*  
123 *alternatus* Hope (Coleoptera: Cerambycidae). *Monochamus alternatus* hosts were collected  
124 from forests in Liyang County, China during the winter of 2011, and maintained at 10°C **for**  
125 **one-two months** before use in experiments. **The foundresses used in the experiment were**  
126 **collected from laboratory colonies where females had been mated with siblings upon**  
127 **emergence. In the treatments with more than one foundress, each foundress was obtained**  
128 **from a different parasitized host.** Laboratory experiments were conducted at 25°C and 60-  
129 80% r.h.

130 The number of adult female *S. harmandi* introduced into a glass vial (1.0cm diameter  
131 × 5.0cm long) with one *M. alternatus* larva was varied (1, 2, 4, 6 or 8). The numbers of eggs  
132 laid onto each host, and the numbers of adult male and female *S. harmandi* offspring  
133 produced, were recorded. There were 220 replicates overall, with between 30 and 60  
134 replicates for each number of foundress females. However, in **about** half of replicates,  
135 foundresses failed to produce any offspring; this was especially common when foundress  
136 group sizes were small and/or hosts were large (Tang et al., 2014), and our current analysis is  
137 restricted to those replicates in which some *S. harmandi* offspring matured. Of the 112  
138 replicates producing surviving offspring, one brood, produced by a single foundress,  
139 consisted of eight males only; probably because the foundress had not mated. Another  
140 offspring group, produced by two foundresses, had an **unusually** large number of males  
141 (19/44 offspring), suggestive that one of the foundresses was unmated. Following procedures  
142 adopted by prior studies of bethylid sex ratios (e.g., Hardy & Cook, 1995), these two  
143 replicates were excluded from the reported analyses. **The** inclusion of the two-foundress  
144 replicate would not have led to any different **conclusion**. There remained 110 offspring  
145 groups for analysis.

146 Data were mainly explored using logistic analysis (generalized linear modelling) in  
147 the Genstat statistical package (version 14.1, VSN International). All statistical testing was 2-  
148 tailed. **Sex ratios were expressed as the proportion of adult offspring that were males. When**  
149 binary data were grouped, we **assumed** quasi-binomially distributed errors to counter the  
150 effects of overdispersion (Wilson & Hardy, 2002). Sex ratio variance and variance in  
151 mortality were **each** quantified using two descriptive statistics, the Heterogeneity Factor (HF  
152 = residual deviance/residual degrees of freedom, West & Herre, 1998) and the variance ratio

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3 153  $R$  (= variance in numbers observed/expected binomial variance, Nagelkerke & Sabelis, 1998)  
4 and analysed using the Meelis test statistic  $U$  which tests for departures from binomial  
5 distribution (significant large negative values indicate underdispersion and large positive  
6 values overdispersion; Nagelkerke & Sabelis, 1998; Krackow et al., 2002). Values of  $R$  and  $U$   
7 are calculated from sums of separate calculations from each offspring group size and we note  
8 that our data consist of small numbers of clutches or broods spread across a large range of  
9 group sizes. Although the approach we adopt is the best currently available, it is known that  
10 when data consist of many small sub-samples estimations can be distorted such that, for  
11 instance, larger values might not correspond to stronger deviations from expected variances  
12 (Krackow et al., 2002).  
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## 21 Results

22 Among the 110 broods in which some offspring matured, overall 61% offspring had died  
23 during development (mean egg-to-adult mortality = 0.614, SE  $\pm$  0.021), and mortality had  
24 variance that was not significantly greater than binomial (HF = 28.3; Meelis test:  $R$  = 12.61,  
25  $U$  = 85.43,  $P$  = 1.00). It is already known that developmental mortality is unrelated to host  
26 weight, the number of eggs laid on a host or the number of foundress females present (Tang  
27 et al., 2014). Unsurprisingly, the size of the offspring group at maturity was negatively  
28 correlated with egg-to-adult mortality (Spearman's rank correlation,  $r$  = -0.757,  $t$  = 12.12, d.f.  
29 = 109,  $P$  < 0.001). The sex ratios of groups of maturing adults were typically strongly female  
30 biased (mean proportion of offspring that were male = 0.069  $\pm$  SE = 0.004). [Note that an  
31 incorrect mean value of 0.032 was reported by Tang et al. 2014]. Sex ratio variances were  
32 overdispersed (HF = 1.48; Meelis test:  $R$  = 0.7208,  $U$  = 2.33,  $P$  = 0.0199).  
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41 Sex ratios decreased weakly with adult offspring group size (Logistic ANCOVA  
42 corrected for overdispersion:  $F_{1,109}$  = 5.55,  $p$  = 0.02, %Deviance explained = 4.75, Figure 1)  
43 and increased weakly with foundresses number ( $F_{4,109}$  = 5.63,  $p$  < 0.001, %Dev = 4.81, Figure  
44 1). There was no significant interaction between these two main effects ( $F_{4,109}$  = 1.08,  $P$  =  
45 0.371). The number of males present in offspring group increased with group size ( $F_{1,108}$  =  
46 80.97,  $P$  < 0.001, %Dev = 42.8%). Sex ratio variance, quantified by HF, was not correlated  
47 with the number of foundresses producing the brood (One,  $n$  = 9, HF = 1.64; Two,  $n$  = 20, HF =  
48 0.816; Four,  $n$  = 31, HF = 1.24; Six,  $n$  = 21, HF = 0.595; Eight,  $n$  = 28, HF = 2.25; Spearman's rank  
49 correlation,  $r$  = 0.100,  $P$  = 0.196). Sex ratios were significantly higher among broods that had  
50 experienced higher proportions of developmental mortality ( $F_{1,108}$  = 10.21,  $P$  = 0.002, %Dev  
51 = 8.63, Figure 2). Sex ratio variance was significantly higher among broods that experienced  
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3 186 the highest percentages of developmental mortality ( $\leq 24.9\%$  mortality,  $n=9$ ,  $HF=0.46$ ; 25-  
4 187 49.9%,  $n=27$ ,  $HF=0.953$ ; 50-74.9%,  $n=42$ ,  $HF=2.08$ ;  $\leq 75\%$ ,  $n=32$ ,  $HF=1.13$ ; Spearman's  
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6 188 rank correlation,  $r=0.800$ ,  $P=0.021$ ).

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8 189 About 15.3% of adult offspring groups consisted of female offspring only. The  
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10 190 probability of an offspring group consisting only of females was lower among larger  
11 191 offspring groups ( $G_1=32.39$ ,  $P<0.001$ ,  $\%Dev=34.0$ ); no broods of more than around 50  
12 192 maturing offspring contained females only (Figure 3). The probability of all-female offspring  
13 193 groups was higher when developmental mortality was higher ( $G_1=20.68$ ,  $p<0.001$ ,  $\%Dev=$   
14 194 21.7) and when foundresses number was lower ( $G_4=5.26$ ,  $P<0.001$ ,  $\%Dev=22.1$ ) (Figure  
15 195 4); the interaction between these two variables was not significant ( $G_4=0.19$ ,  $P=0.945$ ).

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## 21 197 Discussion

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23 198 The highly female biased sex ratios observed in *S. harmandi* appear to be due to LRE (the  
24 199 mutually beneficial foundress-foundress interactions contribute to the value of female  
25 200 offspring, Taylor, 1981) rather than the more usual explanation of LMC (Hamilton, 1967).  
26 201 We first consider the brood compositions observed in *S. harmandi* in regard to current  
27 202 literature and then suggest an approach for how models can be developed to better evaluate  
28 203 the assertion that *S. harmandi* sex ratios have principally evolved due to LRE.

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30 204 Current evidence suggests that *Sclerodermus* foundresses tend to each lay a small  
31 205 number of male eggs in every clutch (Mamaev, 1979; Liu et al., 2011; Tang et al., 2014).  
32 206 Mamaev (1979) reported that 1-2 males are laid among the first produced eggs in each clutch  
33 207 (a pattern which may be associated with final clutch size uncertainty, Hardy, 1992).  
34 208 Similarly, Tang et al. (2014) estimated that an average of 0.8 adult males are produced per *S.*  
35 209 *harmandi* foundress, suggesting that (given 61% developmental mortality) each foundress  
36 210 lays an average of 1.31 male eggs in each clutch. Further, the brood sex ratios produced by  
37 211 single foundresses are typically less female biased than the reciprocal of brood size (one male  
38 212 per brood is expected under single-foundress LMC in the absence of developmental  
39 213 mortality, Green et al., 1982; Griffiths & Godfray, 1988; Nagelkerke & Hardy, 1994), due to  
40 214 mothers laying more males in larger broods than in smaller broods. Weak declines in sex  
41 215 ratio with increasing brood size, as observed in *S. harmandi*, or a lack of relationship between  
42 216 sex ratio and brood size, have been reported for the secondary sex ratios (at adult emergence)  
43 217 of most previously examined sub-social bethylids in which broods are produced by a single  
44 218 foundress and, as observed for *S. harmandi*, are typically associated with an increase in the  
45 219 number of males per brood as brood size increases (Hardy et al., 1998). However,

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3 220 examination of the primary sex ratio (at oviposition) in one sub-social species has shown that  
4 221 developmental mortality may alter or obscure initially present relationships (Khidr et al.,  
5 222 2013). Given that developmental mortality among *S. harmandi* offspring is more common  
6 223 than is observed in many gregarious parasitoids (Hardy et al., 1998; Kapranas et al., 2011),  
7 224 empirical assessment of the primary sex ratio is a desirable future step, especially as such  
8 225 evaluation could potentially also reveal the relative contributions of individual foundresses to  
9 226 each offspring group (Khidr et al., 2014) and thus provide insights into whether the  
10 227 apparently cooperative reproduction observed is in fact tempered by reproductive dominance  
11 228 and skew (Tang et al., 1994).

12 229 Sex ratio variances were overdispersed, most likely due to the scrambling effect  
13 230 developmental mortality has on initially less variable group sexual compositions, as observed  
14 231 within and across other species of gregarious parasitoids (Hardy et al., 1998; Kapranas et al.,  
15 232 2011; Khidr et al., 2013). Theory for sex ratio variance under LRE is not well developed; but  
16 233 under LMC, selection for low sex ratio variance can be less stringent in multiple-foundress  
17 234 cases compared to single-foundress cases (Nagelkerke, 1996); our rather limited data do not  
18 235 suggest a relationship **between** variance and foundress number. Further, selection for sex ratio  
19 236 variance is expected to be related to the frequency at which different numbers of foundresses  
20 237 naturally occur (West & Herre, 1998) but there is very little field **data on** the distribution of  
21 238 foundress numbers in *S. harmandi* populations. The less female biased sex ratios in broods  
22 239 which experienced higher proportions of **mortality** is suggestive of higher mortality among  
23 240 developing females than among males, which may be attributable to female larvae requiring  
24 241 more resources than male larvae to grow large enough for successful pupation (Nagelkerke &  
25 242 Hardy, 1994), and indeed female *S. harmandi* are larger than males (He, 2004), and could  
26 243 also potentially result from sexually-differential care by brood-tending foundresses.

27 244 While the current evidence suggests that developmental mortality increases brood sex  
28 245 ratio variance in *S. harmandi*, there is much stronger evidence for an association between  
29 246 mortality and a related aspect of brood sexual composition: all-female ('virgin') broods,  
30 247 which can result from all males in a brood dying before maturity. Under single-foundress  
31 248 LMC, hymenopteran parasitoids maturing in all-female broods are expected to have low  
32 249 fitness due to being constrained to subsequently produce male-offspring only, and these will  
33 250 have no reproductive opportunities unless some inter-group dispersal and non-local mating  
34 251 occurs (Green et al., 1982; Godfray, 1990; Heimpel, 1994; Nagelkerke & Hardy, 1994; Hardy  
35 252 et al., 1998; Hardy et al., 2005). Virgin females co-founding broods with mated females can  
36 253 obtain fitness via their sons mating locally with the offspring of the co-foundresses, but are



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3 254 not able to produce offspring sex ratios close to the unconstrained optima and are thus also  
4 255 disadvantaged (Godfray, 1990; West, 2009). Despite the disadvantage of virginity, virgin  
5 256 broods are predicted to arise under LMC due to the trade-off between minimising the number  
6 257 of male offspring produced and insuring against all the males dying before maturity (Green et  
7 258 al., 1982; Heimpel, 1994; Nagelkerke & Hardy, 1994; West et al., 1997). In general accord  
8 259 with these expectations from LMC theory, virgin *S. harmandi* broods were more common  
9 260 when brood sizes were smaller, when mortality was more prevalent and also when broods  
10 261 were produced by fewer foundresses (Figures 2, 3, 4). Similar patterns have been observed in  
11 262 gregarious parasitoids with LMC (Hardy et al., 1998; Kapranas et al., 2009, 2011) and across  
12 263 fig wasp species with LMC (West et al., 1997). Further, the relationships between the  
13 264 prevalence of virginity and the prevalence of mortality for *S. harmandi* broods produced by  
14 265 one or two foundresses (Figure 4) resemble explicit predictions for the one- and two-  
15 266 foundress cases under LMC (Figure 1d in West et al., 1997). Whether these apparent matches  
16 267 to the predictions of ‘extended’ LMC theory (West, 2009) counter the assertion (Tang et al.,  
17 268 2014) that ‘classical’ LMC does not explain sex ratios in *S. harmandi* is currently unclear and  
18 269 may require the development of equivalent theory for virginity under LRE.

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30 270 In mixed sex broods, with female biased sex ratios, current evidence suggests that  
31 271 local (within brood) mating is the norm. Given a mean sex ratio of 0.069, individual males  
32 272 will typically mate with around 14 females and evidence from other bethylids suggests that  
33 273 they will have sufficient supplies of sperm with which to successfully inseminate all the  
34 274 females (Hardy et al., 2005), although at high-mating frequencies males are expected (Abe &  
35 275 Kamimura, 2015) and observed (e.g., in the bethylid *Goniozus legneri*, Gordh et al. 1983) to  
36 276 transfer smaller ejaculates. The occurrence of virgin broods suggests that non-local mating,  
37 277 via male dispersal, may also occur, due to the selection pressure on males to acquire more  
38 278 mates (e.g., by immigrating into virgin broods) and that females may be receptive due to the  
39 279 selective advantage for being able to produce female-biased offspring sex ratios. Male *S.*  
40 280 *harmandi* typically possess wings which is suggestive of male dispersal but there is a lack of  
41 281 direct evidence for the mating structure of *Sclerodermus* populations (as with other bethylids;  
42 282 Hardy & Cook, 1995; Hardy & Mayhew, 1998; Hardy et al., 2000).

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51 283 After maturing around the remains of a host, female *Sclerodermus* must disperse to  
52 284 forage for a fresh host. Females typically do not possess wings and so forage by walking. It is  
53 285 not currently known whether females disperse as individuals or as cohesive groups, although  
54 286 reports that females overwinter in groups in host-made tunnels or cavities in trees (Xu et al.,  
55 287 2002; Zhang & Tian, 1985) do suggest group cohesion. The direct selective advantage to

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3 288 cohesive dispersal and foraging would be that it would promote **successful reproduction on**  
4 **large hosts** (Tang et al., 2014). Cohesive dispersal would also enhance the relatedness  
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6 290 between foundresses, which may in turn select for more strongly biased sex ratios (and other  
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8 291 aspects of social behaviour) compared to situations when foundresses were not closely related  
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10 292 (Taylor & Crespi, 1994; Shuker et al., 2004; Abe & Kamimura, 2012; Gardner et al., 2009).  
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12 293 We, however, speculate that females maturing in virgin broods may not disperse as  
13 294 cohesively as females from mixed-sex broods, due to the expected mating opportunities **to** a  
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15 295 virgin foundresses sons being greater when her co-foundresses are not also virgins.

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17 296 In summary, many of patterns in the sexual composition *S. harmandi* broods are  
18 297 similar to those observed in other gregarious, but sub-social, bethylids and also in some  
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20 298 socially solitary species. Sex ratios in these other species are typically interpreted in the  
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22 299 context of LMC theory while, on current evidence, *S. harmandi* sex ratios are better  
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24 300 explained by LRE. This does not preclude that LMC effects are also operating and indeed  
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26 301 they are expected given that within-brood mating occurs. While LMC and LRE are part of the  
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28 302 same theoretical framework (Taylor, 1981), models for sex allocation under LRE are less  
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30 303 extensively developed than are LMC models (West, 2009). We now turn to considering how  
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32 304 best to develop LRE theory to probe the current explanation for sex ratio bias in  
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34 305 *Sclerodermus*.

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36 306 We have suggested that mutually-beneficial cooperative interaction between  
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38 307 foundresses select for female-biased sex allocation through LRE to the extent that a female's  
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40 308 daughters tend to reproduce in close proximity of each other. However, whilst such a  
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42 309 "viscous" population structure can ensure high relatedness between social partners, it can also  
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44 310 lead to intense competition between kin for limiting reproductive resources (Hamilton, 1964),  
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46 311 and this kin competition effect can inhibit or even override the effects of increased  
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48 312 relatedness on sex allocation and other social evolutionary traits (Bulmer, 1986; Frank, 1986;  
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50 313 Taylor, 1988, 1992a; **Gardner et al 2009**; **Rodrigues & Gardner 2015**). Accordingly, a  
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52 314 theoretical analysis is required to establish under which circumstances (if any) limited  
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54 315 dispersal of females may lead to sex ratio bias via LRE. A promising avenue for exploration  
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56 316 will involve models of "population elasticity", whereby the intensity of kin competition is  
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58 317 reduced because extra resources are made available to local populations that are more  
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60 318 cooperative (Taylor, 1992b; Gardner & West, 2006). In the context of *S. harmandi*, these  
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320 319 extra resources may include the larger hosts that can only be successfully parasitized by  
multiple foundresses acting as a cooperative unit.

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3 321 Whilst the extent of female bias under LMC is expected to strongly depend upon the  
4 322 number of foundresses in the breeding group (Hamilton, 1967), the extent of female bias  
5 323 observed in *S. harmandi* does not appear to be strongly dependent upon foundress number  
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8 324 (Tang et al., 2014). Formal theoretical exploration is needed to determine whether this is a  
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10 325 general feature of LRE, or whether this relative invariance owes to a particular feature of *S.*  
11 326 *harmandi*'s biology. Moreover, whilst a "fertility insurance" effect, that curbs the evolution  
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13 327 of extreme female-biased sex ratios in order to ensure the presence of at least some mating  
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15 328 opportunities for daughters in the context of stochastic death or sterility of males, has been  
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17 329 explored in the context of LMC (particularly in relation to protozoan parasites; West et al.,  
18 330 2002; Gardner et al., 2003), the impact of the threat of daughter virginity on a foundress's sex  
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20 331 allocation decision remains to be explored in the context of LRE. The ability of unmated  
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22 332 females to produce male offspring even when unmated adds further complexities for sex ratio  
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24 333 evolution (Godfray, 1990; West, 2009; Gardner, 2014).

25 334 In addition to improving our understanding of the sex allocation of these bethylid  
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27 335 wasps, the proposed model development could also be used to understand the evolution of  
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29 336 their quasisociality, both in terms of the evolution of reduced female dispersal and also in  
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31 337 terms of the evolution of reduced aggressiveness and the emergence of coordinated  
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33 338 cooperation between co-foundresses. In line with Gardner and Ross's (2013) suggestion that  
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35 339 a positive feedback between female-biased sex ratios and female-biased cooperation can  
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37 340 promote eusociality, it would also be useful to assess the potential for such feedbacks to  
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39 341 occur in this quasisocial context. In particular, whilst Gardner and Ross's (2013) model  
40  
41 342 required that some (unexplained) level of female cooperation be present at the outset, the  
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43 343 biology of *S. harmandi* provides a ready explanation for cooperative breeding, i.e., a direct  
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45 344 fitness benefit (Tang et al. 2014).

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47 345 We conclude that considerations of the sex ratios of *S. harmandi* and its quasisocial  
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49 346 congeners are currently constrained to be somewhat heuristic. This is essentially the same  
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51 347 conclusion as reached by recent studies of sex ratios in *Melittobia*, another parasitoid genus  
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53 348 in which similar degrees of sociality are observed (Innocent et al., 2010; Abe et al., 2014).  
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55 349 This further understanding will likely develop best via a combination of field-based  
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57 350 observations, experiments and theoretical modelling.

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## 352 Acknowledgements

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3 353 We thank the late Dr Mark Jervis, who always showed an interest in our work on parasitoids  
4 354 and was always able to suggest connections to other research areas or study systems. He also  
5 355 engaged in several blues jam sessions with A.K. and I.C.W.H., both in Cardiff and in Sutton  
6 356 Bonington, playing both guitar and bass guitar very adeptly. We greatly miss these academic  
7 357 and non-academic interactions with him.

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11 358 We further thank Ling Meng, Fuyan Xu, Xie Chunxia, Zheng Huayin, Xu Ming, Liu Conghui,  
12 359 Wu Shaolong and Richard Wilkinson for their help. This study was supported by the Special  
13 360 Foundation for Agro-Scientific Research in the Public Interest (201103002) and the Natural  
14 361 Science Foundation of China (NSFC-36871670). A.K. was supported by a Marie Curie  
15 362 Fellowship (FP7-PEOPLE-2010-IEF 273431). A.G. was funded by a Natural Environment  
16 363 Research Council (UK) Independent Research Fellowship (NE/K009524/1).

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3 538 **Figure legends**  
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7 **Figure 1.** Relationships between sex ratio (proportion of adult offspring that were males) and  
8 brood size at emergence. Fitted logistic regression lines are provided for each foundresses  
9 group. Note that for visual clarity the fitted logistic regression line for two-foundress broods  
10 is slightly displaced below the four-foundress regression line.  
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15 **Figure 2.** The relationship between sex ratio at adult emergence (proportion males) and the  
16 proportion of developmental mortality in offspring groups. The fitted line is from logistic  
17 regression.  
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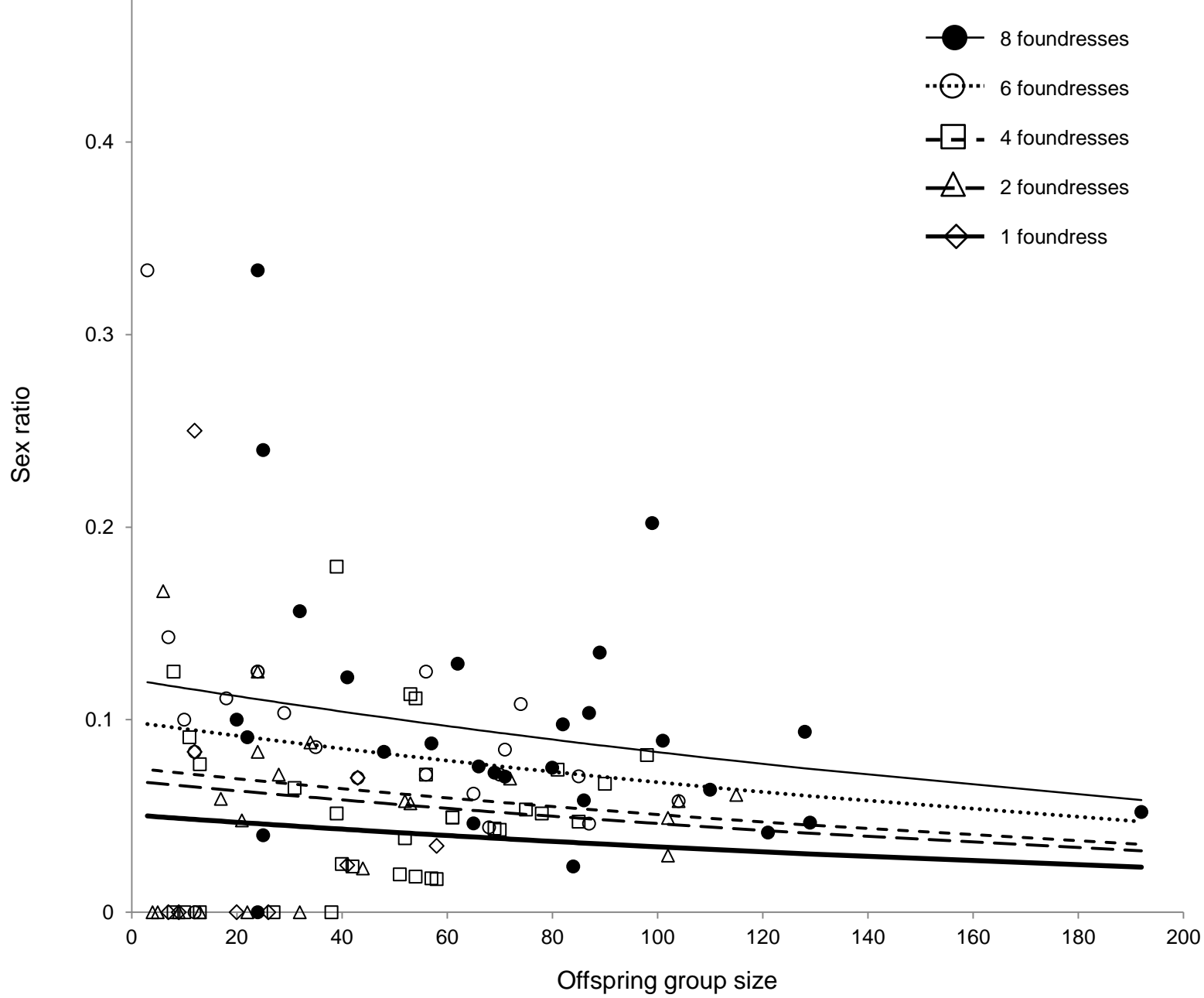
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22 **Figure 3.** The influence of offspring group size at adult emergence on virginity (proportion  
23 of broods that were all-female). The fitted line is from logistic regression.  
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27 **Figure 4.** The influence of foundress number and mortality on virginity (proportion of broods  
28 that were all-female). The fitted lines are logistic analysis of covariance, treating foundress  
29 group size as a discrete factor.  
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Figure 2

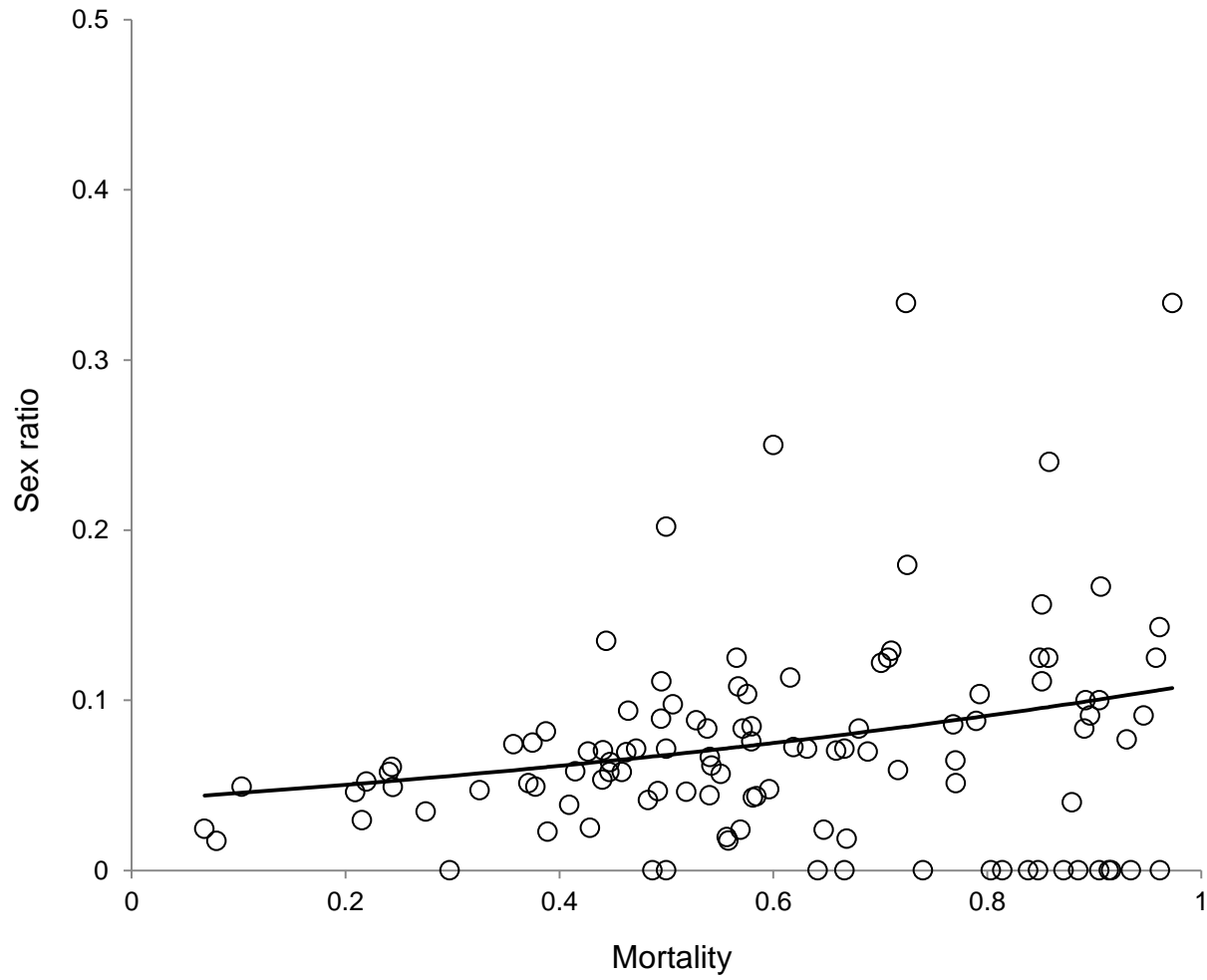
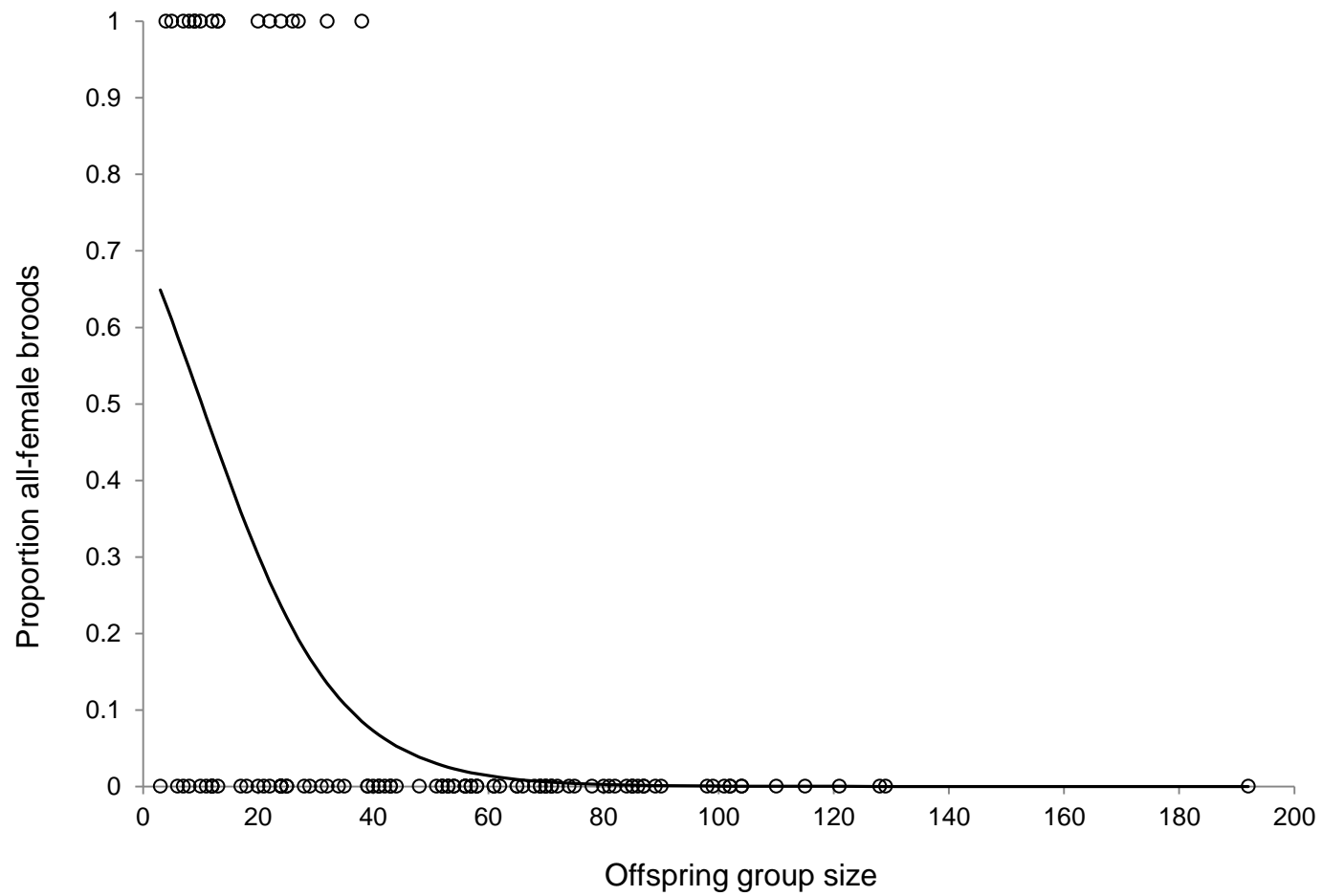
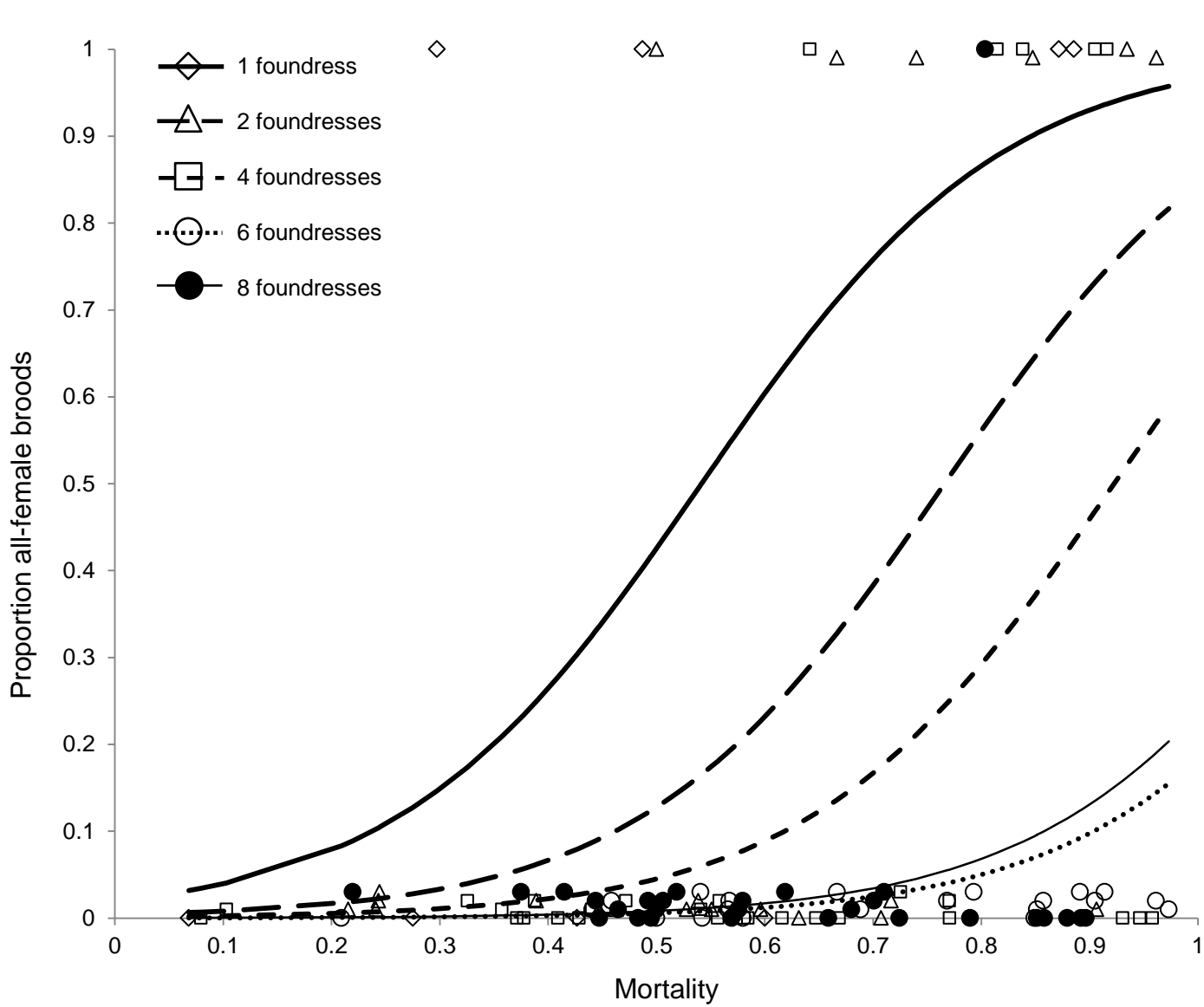


Figure 3



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Figure 4



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