

Co-foundress confinement elicits kinship effects in a naturally sub-social parasitoid

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Abstract

Kinship among interacting individuals is often associated with sociality and also with sex ratio effects. Parasitoids in the bethylid genus *Goniozus* are sub-social, with single foundress females exhibiting post-ovipositional maternal care via short-term aggressive host and brood defence against conspecific females. Due to local mate competition (LMC) and broods normally being produced by a single foundress, sex ratios are female-biased. Contests between adult females are, however, not normally fatal, and aggression is reduced when competing females are kin, raising the possibility of multi-foundress reproduction on some hosts. Here, we screen for further life-history effects of kinship by varying the numbers and relatedness of foundresses confined together with a host resource and also by varying the size of host. We confined groups of 1–8 *Goniozus nephantidis* females together with a host for 5+ days. Multi-foundress groups were either all siblings or all nonsiblings. Our chief expectations included that competition for resources would be more intense among larger foundress groups but diminished by both larger host size and closer foundress relatedness, affecting both foundress mortality and reproductive output. From classical LMC theory, we expected that offspring group sex ratios would be less female-biased when there were more foundresses, and from extended LMC theory, we expected that sex ratios would be more female-biased when foundresses were close kin. We found that confinement led to the death of some females (11% overall) but only when host resources were most limiting. Mortality of foundresses was less common when foundresses were siblings. Developmental mortality among offspring was considerably higher in multi-foundress clutches but was unaffected by foundress relatedness. Groups of sibling foundresses collectively produced similar numbers of offspring to nonsibling groups. There was little advantage for individual females to reproduce in multi-foundress groups: single foundresses suppressed even the largest hosts presented and had the highest per capita production of adult offspring. Despite single foundress reproduction being the norm, *G. nephantidis* females in multi-foundress groups appear to attune sex allocation according to both foundress number and

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foundress relatedness: broods produced by sibling foundresses had sex ratios similar to broods produced by single foundresses (ca. 11% males), whereas the sex ratios of broods produced by nonsibling females were approximately 20% higher and broadly increased with foundress number. We conclude that relatedness and host size may combine to reduce selection against communal reproduction on hosts and that, unlike other studied parasitoids, *G. nephantidis* sex ratios conform to predictions of both classical and extended LMC theories.

KEYWORDS

bethylid, extended local mate competition theory, foundress mortality, *Goniozus nephantidis*, host sharing, offspring production, sex ratio, sociality

1 | INTRODUCTION

Social traits and of sex ratios have been considered in many animal taxa, often simultaneously, with the genetic relatedness between interacting individuals frequently playing a key role (e.g., Agnarsson, Avilés, Coddington, & Maddison, 2006; Avilés, McCormack, Cutter, & Bukowski, 2000; Boomsma & Grafen, 1991; Bourke, 2015; Gardner, Hardy, Taylor, & West, 2007; Helantera, Kulmuni, & Pamilo, 2016; Komdeur, 1996; Quinones, Henriques, & Pen, 2020; Smith, Kapheim, Kingwell, & Wcislo, 2019; Trivers & Hare, 1976; West, 2009). Despite ongoing debate (e.g. Abbot et al., 2011; Birch, 2017; Bourke, 2015), this body of work has contributed substantially to an extraordinary depth of understanding of organismal and social evolution.

Hymenopteran parasitoids have proven to be excellent model systems for testing theories of adaptive sex allocation (e.g., Godfray, 1994; Ode & Hunter, 2002; West, 2009) but there has been relatively little consideration of their sociality. Most parasitoid taxa are socially solitary, but a few exhibit traits associated with advanced sociality. For example, *Copidosoma* wasps in the family Encyrtidae have polyembryonic clonal development and nonreproductive larval castes which defend their reproductive caste kin (Cruz, 1981; Gardner et al., 2007; Giron, Dunn, Hardy, & Strand, 2004; Giron & Strand, 2004; Grbic, Ode, & Strand, 1992), representing reproductive division of labour, but without maternal care. In contrast, species in the bethylid genus *Sclerodermus* have evolved quasi-sociality, in which multiple foundresses parasitize the same host and exhibit cooperative brood care (Abdi, Hardy, Jucker, & Lupi, 2020; Abdi, Lupi, Jucker, & Hardy, 2020; Tang et al., 2014), without apparent division of labour.

Species in the bethylid genus *Goniozus* exhibit a lower level of sociality termed sub-social: individual mothers care for their own offspring, for a period of time immediately after oviposition and until they develop to the pupal stage by remaining on or next to their hosts and aggressively excluding conspecific females from the vicinity (Bentley, Hull, Hardy, & Goubault, 2009; Goubault, Scott, & Hardy, 2007; Hardy & Blackburn, 1991; Venkatesan, Murthy, Rabindra, & Baskaran, 2009). While remaining with the host, females apparently feed on the host haemolymph and carry on maturing

eggs after ovipositing (Humphries, Hebblethwaite, Batchelor, & Hardy, 2006).

A series of experiments (reviewed in Hardy, Goubault, & Batchelor, 2013) has explored factors affecting the outcomes of competitive interactions between pairs of *Goniozus* females (e.g., a brood guarding host 'owner' and an 'intruder') in the context of testing game-theoretic models of contest behaviour (e.g., Enquist & Leimar, 1987; Kokko, 2013). This body of work examined relatively short-term (<3 hr) interactions and considered initial contest outcomes to be decisive, such that losers would be permanently excluded. Although the results are largely compatible with model predications, the possibility of longer-term, or repeated, interactions between competing females remains. Subsequent observations made during microcosm experiments, involving multiple females and multiple hosts confined together during the entire period of offspring development (Sreenivas & Hardy, 2016; M. Velasco Hernández & I.C.W. Hardy, unpublished data), indicate that it is within the behavioural repertoire of females to tolerate each other's presence on a host, even though overall offspring production can be reduced (Legner & Warkentin, 1988; Sreenivas & Hardy, 2016; Venkatesan et al., 2009). Sharing a breeding site is a characteristic of communality, a more advanced level of sociality than sub-sociality, but which does not involve cooperative brood care (Choe & Crespi, 1997; Costa, 2018; Wilson, 1971).

A parallel series of studies has explored factors affecting the sex ratios produced by *Goniozus* females, chiefly in response to the degree of local mate competition (Hamilton, 1967) associated with single foundress reproduction and also to the probability of offspring developmental mortality. These studies have shown that *Goniozus* sex ratios are typically female-biased (usually around 10% of offspring are males) and broadly conform to expectations from theory for the single foundress case (e.g., Green, Gordh, & Hawkins, 1982; Hardy & Cook, 1995; Hardy, Dijkstra, Gillis, & Luft, 1998; Hardy, Pedersen, Sejr, & Linderroth, 1999; Khidr, Mayes, & Hardy, 2012; Luft, 1996). Because adult females typically attempt to exclude each other from individual hosts via aggressive contests, and will also commit infanticide when obtaining a host bearing offspring produced by another female (Bentley et al., 2009; Goertzen &

Doutt, 1975; Goubault et al., 2007; Hardy & Blackburn, 1991), sex allocation responses to variation in foundress number or to foundress relatedness in *Goniozus* species have been little considered.

Here, we utilize naturally sub-social *Goniozus* wasps to explore the life-history effects of three factors, host size, foundress number and foundress relatedness, that may have influenced the evolution of both sociality and sex allocation in bethylids and perhaps in other parasitoids. Taxonomically, most hymenopterans that have parasitoid life histories are placed within the Parasitica but bethylids are placed within the Aculeata. Bethylids are members of the Chrysidoidea, the sister group of other aculeate taxa in which eusociality has evolved (Peters et al., 2017) and in which the interplay between sex ratio and sociality has hitherto been most studied (e.g., Boomsma & Grafen, 1991; Bourke, 2015; Helantera et al., 2016; Quinones et al., 2020; Smith et al., 2019; Trivers & Hare, 1976).

In terms of parasitoid sociality, first, host size appears to be correlated with bethylid sociality. The socially solitary bethylid *Laelius pedatus* attacks small hosts (~1.5 mg) and lays clutches of 1–6 eggs (Klein, Ballard, Lieber, Burkholder, & Beckage, 1991; Mayhew, 1998; Mayhew & Godfray, 1997; Mayhew & Hardy, 1998; Mayhew & Heitmans, 2000). Sub-social species of *Goniozus* oviposit onto medium-sized hosts (~35 mg) with clutches ranging up to approximately 18 eggs (Hardy, Griffiths, & Godfray, 1992; Sreenivas & Hardy, 2016). Quasi-social species of *Sclerodermus* can attack large hosts (up to ~250 mg) and lay clutches of more than 100 eggs (Wei, Tang, Wang, Cao, & Yang, 2014). Evolutionary transitions between solitary and sub-social behaviour may be due to only medium- or large-sized hosts being worth the cost of brood defence (Hardy & Blackburn, 1991), whereas transitions to quasi-sociality are likely favoured by the inability of individual females to suppress and exploit very large hosts (Abdi, Hardy, et al., 2020; Abdi, Lupi, et al., 2020; Liu, Xu, Li, & Sun, 2011; Tang et al., 2014; Wei et al., 2014).

Second, across all parasitoid wasps, larger numbers of females attempting to exploit a host are usually associated with greater competition which, in gregarious species, typically reduces clutch sizes produced by each female (Godfray, 1994; Visser, 1996) or, as in *Goniozus*, may lead to the aggressive exclusion of some females from the host resource and subsequent brood defence (maternal care) (Hardy et al., 2013; Humphries et al., 2006; Petersen & Hardy, 1996). However, multiple foundresses can also exhibit cooperation in host exploitation: cooperation between foundresses of the quasi-social *Sclerodermus* results in direct (per foundress) fitness benefits when hosts are large (Tang et al., 2014).

Third, there is a substantial literature on the role of kinship (genetic relatedness) in the evolution of social behaviour and sociality (e.g. Abbot et al., 2011; Birch, 2017; Bourke, 2014; Gardner et al., 2007; Hamilton, 1964a, b; Queller, 2000; Quinones et al., 2020). Kin selection theory predicts that individuals will tend to show less aggression and more altruism towards relatives (Hamilton, 1963, 1964a). Kin recognition abilities have been found in many animal species; among parasitoid hymenopterans, kinship has been found to mediate intra-clonal attack by the soldier caste in polyembryonic encyrtids (Dunn, Dunn, Strand, & Hardy, 2014; Giron et al., 2004; Giron

& Strand, 2004), the aggressiveness of female–female contests in the socially solitary Eupelmid *Eupelmus vuilletti* (Mathiron, Pottier, & Goubault, 2019) and the sub-social *Goniozus legneri* (Lizé, Khidir, & Hardy, 2012) and, most recently, the time to host attack by groups of quasi-social *Sclerodermus* foundresses (Abdi, Hardy, et al., 2020; Abdi, Lupi, et al., 2020).

In terms of sex allocation, all of the above-mentioned social parasitoid taxa experience group-structured mating among their offspring with consequent selection for female bias, whether due to local mate competition (LMC) or local resource enhancement (LRE) (Gardner et al., 2007; Grbic et al., 1992; Hamilton, 1967; Hardy & Cook, 1995; Tang et al., 2014; Taylor, 1981; West, 2009).

First, host size may correlate with sex ratios via brood size effects if larger broods are produced on larger hosts (Hardy et al., 1992) and the numbers of males per brood remain relatively constant (as observed in some but not all gregarious species, e.g., Hardy et al., 1998; Hardy et al., 1999).

Second, classical LMC theory predicts that sex ratio optima for individual foundresses contributing offspring to a mating group will vary according to the number of other foundresses contributing; sex ratio bias is selected for when foundress numbers are low due to a reduction in competition between related males for mates and due to enhanced mating opportunities for males (Hamilton, 1967, 1979; Taylor, 1981; West, 2009).

Third, extensions to classical LMC theory have predicted that selection will favour greater female bias when closer kin interact, such as when foundresses that share a patch can assess their relatedness and have shared genetic interests in reducing mate competition among males (Burton-Chellew et al., 2008; Frank, 1985; Taylor & Crespi, 1994; West, 2009).

To evaluate the effects of host size, foundress number and relatedness on sex ratios and sociality, we confine *Goniozus nephantidis* (Muesebeck) females (experimentally varying number and relatedness) together with one host (experimentally varying size). We aim to discover whether prolonged female–female contact elicits behavioural and reproductive responses (such as foundress mortality, host attack, offspring production, offspring size and sex ratio) that are commensurate with those observed in species with naturally higher foundress numbers or levels of sociality. Hence, using a heuristic approach, we force sub-social parasitoids that normally reproduce as single foundresses into a range of conditions that probe their incipient propensity to transition to communal, or even quasi-social, reproduction and to respond to foundress number and kinship in the direction predicted by sex ratio theory.

Our key expectations are that larger hosts may be more difficult for foundresses to suppress but, once suppressed, will provide greater resources and thus enhance offspring production, whether in terms of offspring size or number. We also expect that greater number of foundresses will be associated with enhanced host suppression but also subsequently increased resource competition and, thus, higher foundress mortality and reduced per-foundress reproductive output. From classical LMC theory, we expect that offspring group sex ratios will become less female-biased as the number of

foundresses increases. For relatedness, we expect that closer kinship among multiple foundresses will be associated with lower foundress mortality, increased host sharing and enhanced reproductive output. From extended LMC theory, we expect that sex ratios will be more female-biased when foundresses are more closely related. As effects of host size, foundress relatedness and number may occur additively, synergistically or antagonistically, we also evaluate their interactions.

2 | MATERIALS AND METHODS

2.1 | Insects

The natural hosts of *G. nephantidis* are the mid-to-late larval stages of *Opisina arenosella* Walker (Lepidoptera: Oecophoridae), a defoliator of coconut in the Indian sub-continent (Cock & Perera, 1987). Although it appears to be monophagous in the field (Shameer, Nasser, Mohan, & Hardy, 2017), *G. nephantidis* can be laboratory-reared on the rice moth *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) and the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Galleridae): developmental parameters, such as clutch size, adult offspring production and sex ratio, are similar whether the natural host or either of these factitious hosts is utilized (Mohan & Shameer, 2003). Our *G. nephantidis* stock had been maintained in laboratory culture reared for over 10 years on *C. cephalonica* but in our experiments, we used a culture of *Ga. mellonella*, obtained from a local pet food retailer, as the only host species because we wished to assess the influence of a wide range of host sizes on parasitoid behaviour: *Ga. mellonella* larvae commonly reach >500 mg before pupation, whereas *C. cephalonica* larvae >90 mg are unusual (Hardy et al., 1992).

Both *C. cephalonica* and *Ga. mellonella* were reared on a diet of wheat bran, fine corn meal, brewer's yeast granules, honey and glycerol (Sreenivas & Hardy, 2016). All cultures and experimental replicates were kept in a climate room at the University of Nottingham, maintained at 26°C, 18L:6D and 60%–80% RH.

2.2 | Experiment

We evaluated whether foundress mortality, foundress behaviour and offspring production were affected by the number of foundresses, the relatedness between foundress females and the size of the host. *Galleria mellonella* hosts from a wide range of weights (measured using a digital microbalance to an accuracy of 0.0001 g) were selected and placed individually into glass vials (1.0 cm diameter × 5.0 cm long) stoppered with nylon gauze and cotton wool. Adult *G. nephantidis* females (foundresses) were then added into the vials: there were replicates with 1, 2, 3, 4, 6 and 8 foundresses. Within each multi-foundress replicate, the foundresses were added simultaneously. The relatedness between foundresses in multi-foundress replicates was also varied, such that either all foundress were

brood-mate sisters that had developed on the same host (siblings) or all had developed in different broods within the culture (nonsiblings). To give adequate overall statistical power (Smith, Hardy, & Gammell, 2011), we set up 20 replicates of each foundress number and relatedness combination ([20 replicates × 6 foundress number treatments × 2 relatedness treatments] – 20, due to foundress relatedness being invariant in single foundress replicates, = 220 replicates overall). We ensured that a wide range of host sizes was represented within the 20 replicates for each foundress number and relatedness combination. This experimental design thus followed that of Tang et al. (2014), studying the quasi-social bethylid *Sclerodermus harmandi*, but with more foundress number treatments and the addition of variation in foundress relatedness.

The parasitoids and hosts within each replicate were examined once daily. We recorded the number of foundresses alive, the paralysis of the host (cessation of major locomotory activity), whether foundresses were positioned on or next to the host or where positioned further away from the host (within the confines of the vial), the occurrence of oviposition (the first observation of clutches of eggs on hosts' integuments), the number of eggs laid (total observed within each replicate), the duration of offspring development (from the first day an egg was observed on the surface of a host to the first day an adult offspring emerged) and the numbers of adult male and female offspring produced. Comparisons of the numbers of adult offspring and the number of eggs laid allowed us to calculate the proportions of offspring that died during development. We also recorded the average size of female offspring produced (a correlate of fitness in *G. nephantidis* and other parasitoids; Hardy et al., 1992; Godfray, 1994; Petersen & Hardy, 1996) within each offspring group. The thorax length of each adult female was measured under a binocular microscope fitted with an ocular micrometre.

2.3 | Statistical analysis

The influences of foundress number, foundress relatedness and host size on foundress mortality, behaviour, offspring production and sex allocation were explored using generalized linear modelling (Crawley, 1993; Faraway, 2006) in the *GenStat* statistical package (v17.1, VSN International Ltd.). Foundress relatedness in multi-foundress replicates was treated as a factor with two levels (siblings or nonsiblings). Foundress number was fitted as a factor in most statistical analyses but as a continuous variable in some sex ratio analyses to assist comparison with predictive theory. Host weight was fitted as a continuous explanatory variable except in time-to-event data analysis, such as time to paralysis and time to oviposition, in which host size was categorized as small, medium or large. First-order interactions between main effects were included in the initial statistical models. Hypothesis testing was carried out using backwards elimination of explanatory variables from initial models and by aggregation of factor levels to find minimum adequate statistical models (Crawley, 1993; Wilson & Hardy, 2002). In analyses of effects of inter-foundress relatedness, we excluded single

foundress replicates. In log-linear and logistic analysis, of small integer and proportional response variables, respectively, under- and over-dispersion was taken into account, where appropriate, via empirical estimation of scaling parameters and thus the assumption of quasi-Poisson and quasi-binomial error distributions (Crawley, 1993; Wilson & Hardy, 2002). Following logistic and log-linear analyses, we give the percentage deviance explained (%Dev) as a descriptor analogous to r^2 .

Time-to-event data on host paralysis and oviposition were analysed using parametric cohort survival analyses with censoring (Aitkin, Anderson, Francis, & Hinde, 1989; Crawley, 1993). We first compared exponential models (constant hazard function) against Weibull models (time-dependent hazard function): in all cases, the Weibull models provided a significantly better description of the data. The influences of the candidate explanatory factors (see above) were then explored by their inclusion into the Weibull models (Aitkin et al., 1989; Crawley, 1993).

As we performed over 100 statistical hypothesis tests, we used the false discovery rate (FDR) procedure to control for multiple

comparisons (Benjamini & Hochberg, 1995; McDonald, 2014). We treated results presented in Table 1 as a family of tests (including evaluations of interactions, Cramer et al., 2016) and set the family-wide α -value to 0.10. The interpretation of two results was altered by this procedure.

3 | RESULTS

Table 1 provides an overview of results presented in this section.

3.1 | Foundress mortality

Two of the 20 single foundresses died by the end of the fifth day after host presentation. Similarly, 11.30% (104/920) of foundresses in multi-foundress groups died during this period. Mortality was more common among foundresses in nonsibling groups than among siblings, and this difference increased with time (Figure 1) and was

TABLE 1 Summarized key results

Reproductive trait	Foundress number (FN)	Foundress relatedness (FR)	Host size (HS)	Significant interactions
Foundress mortality	Increased with FN	Less when FR higher	Decreased with HS	Lower among larger FN when FR high
Proportion of hosts paralysed	Increased with FN	NS	NS	
Number of foundress on host	Increased with FN*	NS	Increased with HS	
Proportion of foundress on host	NS	NS	Increased with HS	
Oviposition probability	Increased with FN	Significant via interaction	Increased with HS	Higher for larger FN when FR high; lower when FR low Low for large FN when HS small
Time to oviposition after host presentation	Significant via interaction	Significant via interaction	Significant via interaction	Earlier when FN larger and FR high Earlier when FN larger and HS smaller
Time to oviposition after paralysis	Significant via interaction	Significant via interaction	Significant via interaction	Earlier when FN larger and FR high Earlier when FN larger and HS smaller
Clutch size ^a	Increased with FN	Larger when FR higher*	Increased with HS	
Eggs laid per foundress	Decreased with FN	NS	Increased with HS	
Developmental time	Increased with FN	NS	Decreased with HS	Earlier when FN lower and HS larger
Developmental mortality	Increased with FN	NS	Decreased with HS	
Total brood size	HS-dependent effect	NS	FN-dependent effect	For small FN, increased with HS; for large FN decreased
Per-foundress brood size	HS-dependent effect	NS	FN-dependent effect	For small FN increased with HS; for large FN decreased
Female offspring size	NS	NS	Increased with HS	
Female-biased sex ratio ^a	Decreased with FN	Greater when FR high	NS	
Males per brood	NS	Fewer when FR high	NS	
Females per brood	Significant via interaction	More when FR high	Significant via interaction	For small FN increased with HS; for large FN decreased

Abbreviation: NS, no statistically significant influence detected.

^aOutlier excluded.

*Significant effect that became nonsignificant after multiple comparison correction using a false discovery rate of 0.10.

significant from the first day through to the fifth day (cumulative mortality: First: $G_1 = 15.32$, $p < .001$, %Dev = 7.32; Second: $G_1 = 12.21$, $p < .001$, %Dev = 5.99; Third: $G_1 = 17.11$, $p < .001$, %Dev = 8.11; Fourth: $G_1 = 2,817$, $p < .001$, %Dev = 12.68; Fifth: $G_1 = 34.16$, $p < .001$, %Dev = 14.97).

The probability of mortality (by the end of day 5) was also higher when there were more foundresses ($G_5 = 4.35$, $p < .001$, %Dev = 8.87) and was lower when hosts were larger ($G_1 = 15.78$, $p < .001$, %Dev = 6.44). There was no interaction between foundress number and host size ($G_5 = 0.71$, $p = .615$, %Dev = 1.45) or between relatedness and host size ($G_4 = 1.97$, $p = .162$, %Dev = 0.66) but there was an interaction between foundress size and relatedness ($G_4 = 4.36$, $p = .002$, %Dev = 5.87) such that mortality in larger foundress groups was reduced when foundresses were siblings. Considering the amount of resource available per foundress (host weight/initial number of foundresses) showed that foundress mortality was entirely absent when resources were relatively abundant (>0.1 g per foundress) and was more common among nonsiblings than among siblings when resources were more limiting (Resource availability: $G_1 = 35.43$, $p < .001$, %Dev = 13.23; Relatedness: $G_1 = 35.43$, $p < .001$, %Dev = 13.23; Figure 2). The causes of foundress mortality could not be identified but in some replicates, small hosts died and foundress mortality followed. We also observed female–female contests in at least one replicate of each multiple foundress treatment combination.

3.2 | Probability of paralysis

Almost all (98.18%) hosts became paralysed and all the hosts that were paralysed were paralysed within one day. The probability of

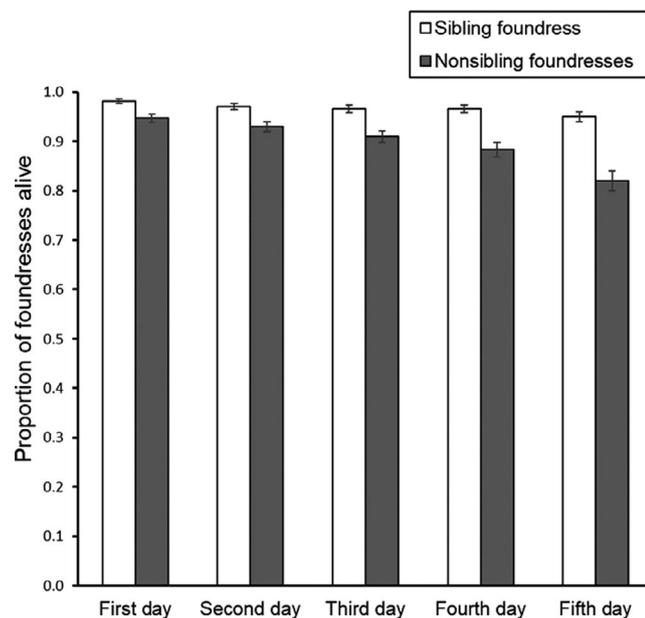


FIGURE 1 Increasingly apparent effects of co-foundress relatedness on cumulative foundress mortality over the first five days of confinement. The effect of relatedness was significant from the first day onwards. Error bars show ± 1 SEM

paralysis was greater when there were more foundresses ($G_5 = 2.22$, $p = .049$, %Dev = 27.76) and successive aggregation of factor levels showed that host paralysis was similarly likely (overall 93.33%) when there were one or two foundresses, whereas larger numbers of foundresses always paralysed the host. However, the effect of foundress number on host paralysis became nonsignificant after correction for multiple comparisons (Table 1). We observed simultaneous attack of the host by multiple foundresses in sibling and in nonsibling experimental treatments. The probability of paralysis was not affected by foundress relatedness ($G_1 = 0.00$, $p = .967$, %Dev = 0.01) or host size ($G_1 = 0.00$, $p = .999$, %Dev = 0.00) or by any interactions (Foundress number \times Relatedness: $G_4 = 0.00$, $p = 1.00$; Foundress number \times Host size: $G_4 = 0.00$, $p = 1.00$; Foundress relatedness \times Host size: $G_1 = 3.04$, $p = .081$).

3.3 | Position of foundresses

Although aggressive contests were observed in all multi-foundress treatments (see above), we also observed multiple females simultaneously present on single hosts, indicating that individual females did not always exclude all other females from the vicinity of the host. The mean number of foundresses in close spatial association with (on or next to) a host was 2.646 ($SE = +0.128$, -0.122 ; data analysed were the per-replicate means from the five daily observations). The mean number of foundresses observed on a given host increased with the number of foundresses in the replicate ($F_{5,218} = 100.83$, $p < .001$, %Dev = 67.06, Figure 3a) and with host size ($F_{1,214} = 47.43$, $p < .001$, %Dev = 6.31) but was not influenced by relatedness ($F_{1,195} = 3.49$, $p = .063$, %Dev = 0.70) or by interactions between these variables (Foundress number \times Relatedness: $F_{4,194} = 1.68$, $p = .157$, %Dev = 1.35, Foundress number \times Host size: $F_{5,113} = 1.68$, $p = .141$, %Dev = 1.12; Relatedness \times Host size: $G_1 = 0.36$, $p = .549$, %Dev = 1.17). The mean proportion of foundresses closely associated with hosts was 0.615 ($SE = +0.0324$, -0.0334) and increased with an increase of host size ($G_1 = 9.14$, $p = .002$, %Dev = 15.83, Figure 3) but was not influenced by foundress number ($G_4 = 0.34$, $p = .848$, %Dev = 3.26) relatedness ($G_1 = 0.11$, $p = .739$, %Dev = 0.26) or by any interactions (Foundress number \times Relatedness: $G_4 = 0.49$, $p = .745$, %Dev = 4.61; Foundress number \times Host size: $G_5 = 1.00$, $p = .415$, %Dev = 8.67; Relatedness \times Host size: $G_1 = 0.08$, $p = .772$, %Dev = 0.20).

3.4 | Probability of oviposition

Of the 216 of hosts that were paralysed, 93.52% were subsequently oviposited on. The probability oviposition was greater when hosts were larger ($G_1 = 20.39$, $p < .001$, %Dev = 19.67) and when there were more foundresses ($G_5 = 3.67$, $p = .003$, %Dev = 17.71). There was also a significant interaction between host size and foundress number ($G_1 = 16.65$, $p < .001$, %Dev = 16.06), as some large groups of foundresses did not oviposit when hosts were very small but

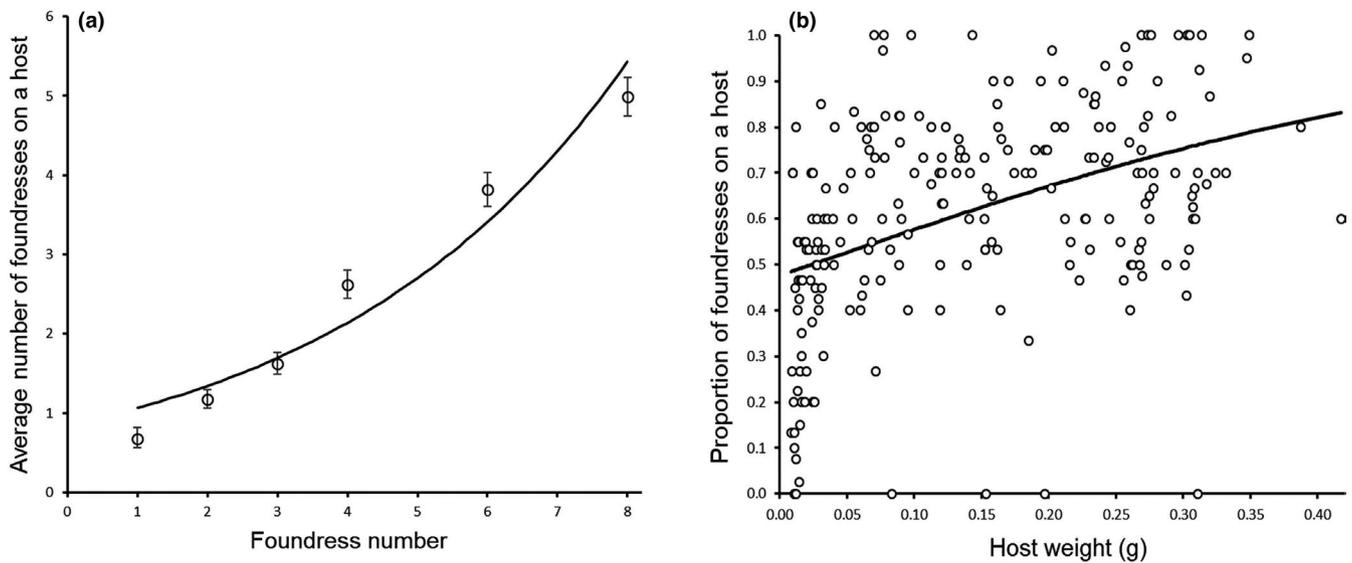


FIGURE 2 Effects of foundress relatedness and resource availability on foundress mortality by the end of the fifth day of confinement. Single foundress group is not significantly different from either sibling or nonsibling groups, but the latter two were different from each other

otherwise always laid eggs, whereas for smaller groups of foundresses, the probability of oviposition was less affected by host weight.

Excluding single foundress replicates and thus considering foundress relatedness found a significant interaction between foundress number and relatedness (Relatedness main effect: $G_1 = 0.31$, $p = .579$, %Dev = 0.31; Interaction: $G_4 = 3.52$, $p = .007$, %Dev = 13.91, $n = 198$), such that the probability of oviposition increased with an increasing foundress number for siblings but decreased for nonsiblings (Figure 4). There was no significant interaction between foundress relatedness and host size ($G_1 = 1.79$, $p = .181$, %Dev = 1.77).

3.5 | Timing of oviposition

Among replicates in which there was oviposition ($n = 202$), the time from the presentation of a host to the foundress(es) to the first laying of eggs ranged between 1 and 8 days. In some multiple foundress replicates, we observed eggs being laid in temporally separate batches, often with the first batch failing to mature before a second batch was laid. Times to begin oviposition were affected by an interaction between foundress number and host size (from host presentation: $G_{10} = 109.9$, $p < .001$, %Dev = 33.30; from host paralysis: $G_{10} = 107.7$, $p < .001$, %Dev = 33.82; Host size main effect: from presentation: $G_2 = 0.7$, $p = .705$, %Dev = 0.138; from paralysis: $G_2 = 2.4$, $p = .301$, %Dev = 0.596; Foundress number main effect: from presentation: $G_5 = 11.0$, $p = .051$, %Dev = 2.599; from paralysis: $G_5 = 7.5$, $p = .186$, %Dev = 1.813); times were shorter when hosts were smaller and there were more foundresses (Figure 5).

Excluding single foundress replicates and considering foundress relatedness found that times to oviposition were not affected by an interaction between relatedness and host size (from presentation: $G_2 = 4.4$, $p = .111$, %Dev = 1.76; from paralysis: $G_2 = 4.5$, $p = .105$, %Dev = 0.241) but were affected by an interaction between foundress

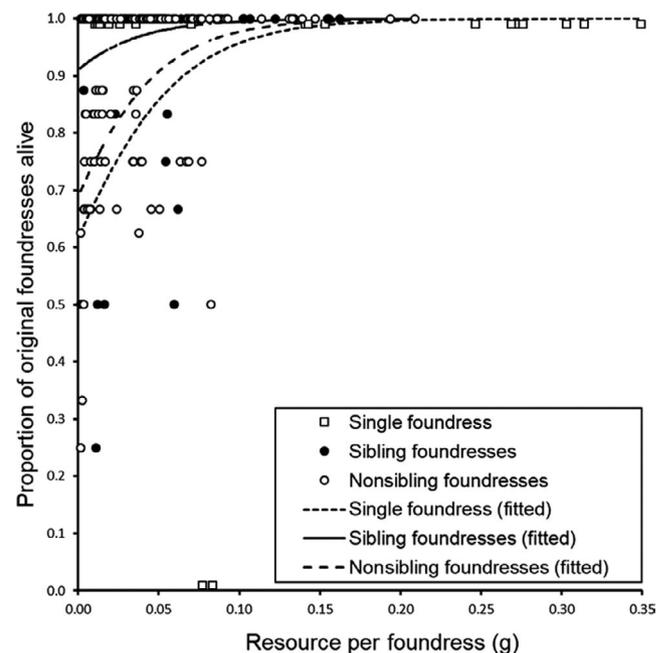


FIGURE 3 Wasps sharing hosts. (a) The relationship between foundress number and the mean number of foundresses observed on or next to the host. Line fitted by log-linear regression. Error bars show ± 1 SEM. (b) The relationship between the proportion of foundresses on a host and the size of the host. Line was fitted by logistic regression. In both panels, data were analysed as the mean across 5 days for each replicate

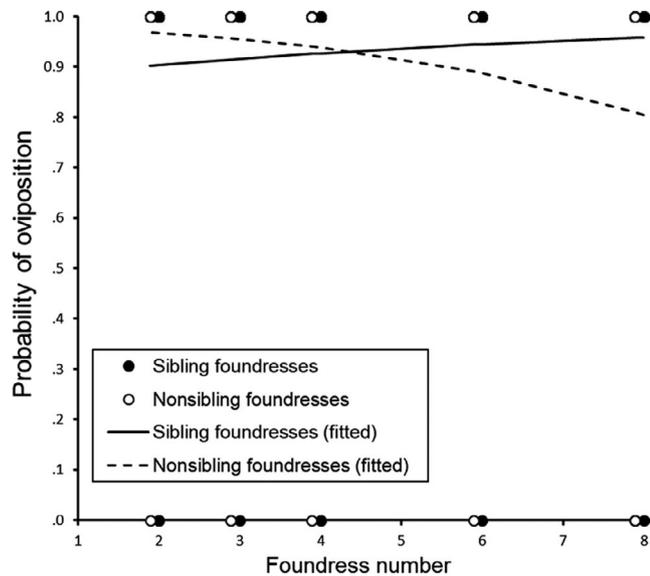


FIGURE 4 The effect of foundress number and relatedness on the probability of oviposition

relatedness and number (from presentation: $G_4 = 12.6$, $p = .013$, %Dev = 3.878; from paralysis: $G_4 = 13.0$, $p = .011$, %Dev = 2.761; Relatedness main effect: from presentation: $G_1 = 0.0$, $p = 1.000$, %Dev = 0.000; from paralysis: $G_1 = 0.1$, $p = .752$, %Dev = 0.000), such that times to egg laying were shorter when foundresses were siblings and there were more foundresses (Figure 5).

3.6 | Clutch size

Clutches laid by single foundresses ranged from 6 to 33 eggs and clutches laid by multiple foundress groups ranged up to 72: this maximum was observed from eight sibling foundresses, whereas clutch size did not exceed 51 in other replicates. As noted above, several temporally separate, clutches were laid on some hosts. Here, we consider clutch size as the total number of eggs observed within a replicate. Clutch size increased with an increase of both host size ($F_{1,196} = 80.19$, $p < .001$, %Dev = 24.70) and foundress number ($F_{5,200} = 8.51$, $p < .001$, %Dev = 13.10), without a significant interaction ($F_{5,195} = 1.90$, $p = .097$, %Dev = 2.92, $n = 202$, Figure 6a).

Analysis of multiple foundress replicates only ($n = 184$) confirmed that clutch size was positively affected by host size ($F_{1,174} = 73.46$, $p < .001$, %Dev = 24.87) further found that it was affected by an interaction between foundress number and relatedness (Number: $F_{4,177} = 9.24$, $p < .001$, %Dev = 12.51; Relatedness: $F_{1,174} = 4.17$, $p = .043$, %Dev = 1.41; Interaction: $F_{4,173} = 2.59$, $p = .039$, %Dev = 3.51); on hosts of a given size, larger clutches were laid by larger groups of foundresses and also by sibling groups (Figure 6b) but the clutch size response of nonsibling foundresses groups was less than that of sibling groups. The interaction between foundress relatedness and host size was not significant ($F_{1,169} = 0.10$, $p = .749$, %Dev = 0.04).

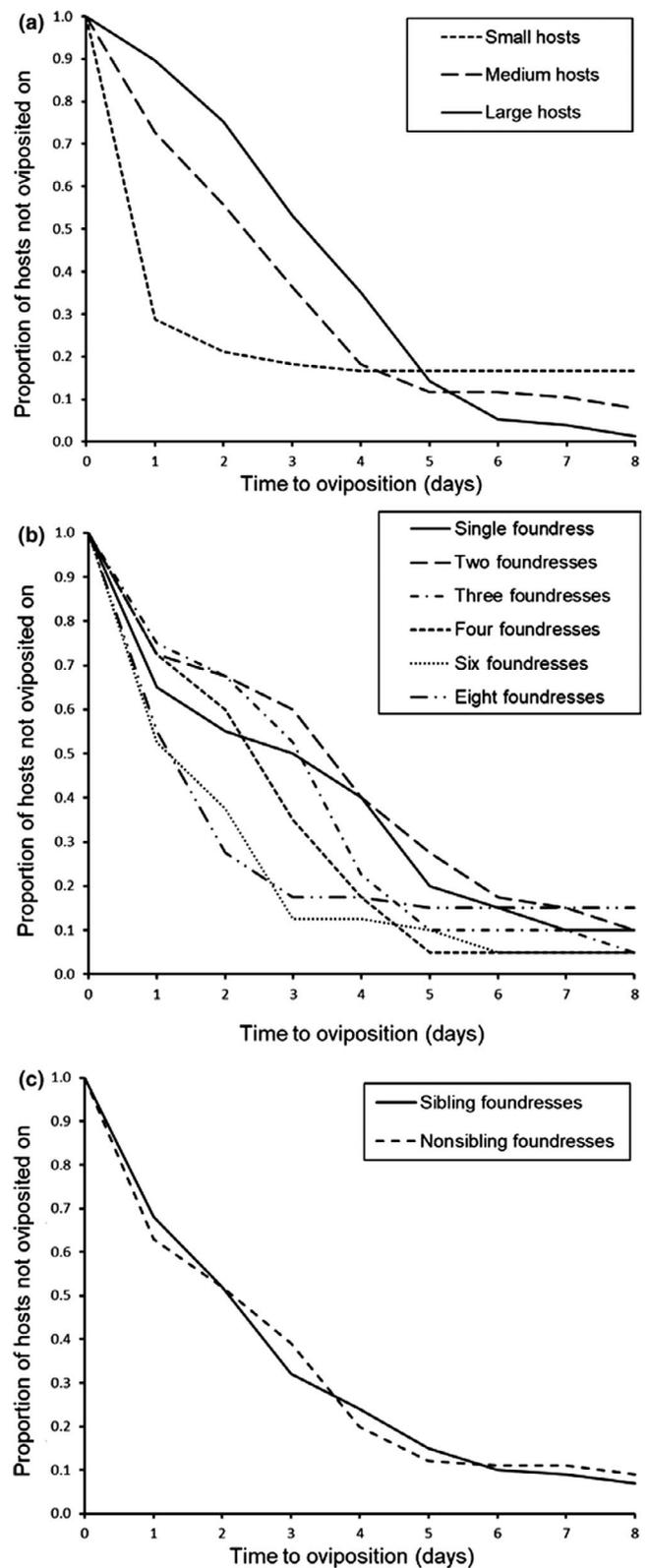


FIGURE 5 Influences on time from host presentation to oviposition. (a) Host size; (b) foundress number; (c) foundress relatedness. Panels show main effects separately: host size and foundress number also interacted significantly, as did foundress number and relatedness

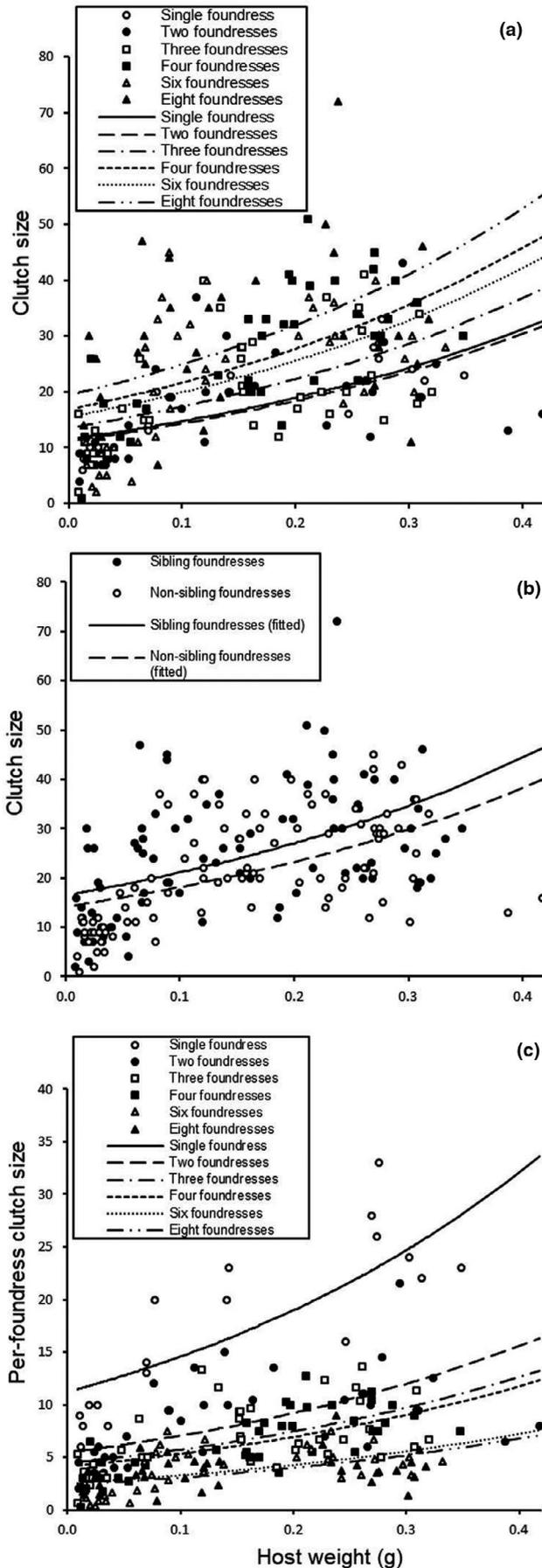


FIGURE 6 Effects of host weight, foundress number and relatedness on clutch size. Panel (a) shows effects of host weight and number of foundresses on the number of eggs laid on to hosts, whereas panel (b) illustrates the possible effects of foundress relatedness on clutch size. Panel (c) shows effects of host weight and number of foundresses on the mean per capita offspring production for foundresses

As the clutch produced by siblings in one replicate was exceptionally large (see above) and could have a disproportionate influence on data interpretation, we re-tested the effect of relatedness on clutch size with this replicate removed: this confirmed that larger clutches were produced by sibling foundresses ($F_{1,169} = 4.22, p = .042, \%Dev = 1.48, n = 183$) but the interaction between foundress number and relatedness was no longer significant ($F_{4,176} = 2.07, p = .086, \%Dev = 2.90$) and nor was the interaction between foundress relatedness and host size ($F_{1,168} = 0.23, p = .633, \%Dev = 0.08$). Further, the effect of foundress relatedness on clutch size became nonsignificant after correction for multiple comparisons (Table 1): current data thus do not allow firm conclusions regarding effects of foundress relatedness on clutch size.

The mean number of eggs laid per foundress (clutch size divided by number of foundresses) was not influenced by relatedness (multiple foundress replicates: $F_{1,178} = 1.07, p = .302, \%Dev = 0.29$) or by its interaction with foundress number ($F_{4,177} = 1.84, p = .124, \%Dev = 2.01$) or by an interaction with host size ($F_{1,169} = 0.02, p = .890, \%Dev = 0.01$). Across all foundress number treatments, per-foundress clutch size increased with host size ($F_{1,196} = 112.99, p < .001, \%Dev = 17.70$) and decreased progressively with increase in foundress number ($F_{5,200} = 63.90, p < .001, \%Dev = 50.06$), without significant interaction between host size and foundress number ($F_{5,195} = 1.64, p = .152, \%Dev = 1.28$): per capita production by foundresses was greatest when females reproduced alone (Figure 6c).

3.7 | Developmental time

The time taken for eggs to develop to adulthood time ranged from 11 to 25 days. Development was not influenced by foundress relatedness (analysis of multiple foundress replicates only: $G_1 = 0.7, p = .403, \%Dev = 0.25, n = 184$). There was no significant interaction between foundress relatedness and host size ($G_2 = 0.3, p = .861, \%Dev = 0.13$). The interaction between foundress relatedness and number was not significant ($G_4 = 9.20, p = .056, \%Dev = 2.27$). Developmental time was affected by host size, foundress number and their interaction (Host size: $G_2 = 49, p < .001, \%Dev = 12.72$; Foundress number: $G_5 = 54.6, p < .001, \%Dev = 16.03$; Interaction: $G_{10} = 38.5, p < .001, \%Dev = 11.06$), such that development was faster on medium or larger hosts, and in replicates with progressively fewer foundresses.

3.8 | Developmental mortality

The overall probability of offspring pre-adult mortality was 0.6236 ($SE = +0.0261, -0.0269$). We observed several instances of entire

clutches of eggs failing to reach the larval stage, with subsequent oviposition of a further clutch of eggs. Overall, developmental mortality was not affected by relatedness (multiple foundress replicates only, $n = 184$: $F_{1,178} = 1.80$, $p = .182$, %Dev = 0.82) or by its interaction with foundress number ($F_{4,173} = 0.37$, $p = .829$, %Dev = 0.68) or host size ($F_{1,169} = 0.40$, $p = .526$, %Dev = 0.18). Across all replicates ($n = 202$), developmental mortality decreased as host size increased ($F_{1,196} = 11.35$, $p < .001$, %Dev = 4.35) and increased as foundress number increased ($F_{5,200} = 10.81$, $p < .001$, %Dev = 20.72) without significant interaction ($F_{5,195} = 1.54$, $p = .178$, %Dev = 2.96; Figure 7).

3.9 | Brood size

The total number of adult offspring produced from hosts that had been oviposited on ranged between 1 and 38 (mean = 11.6023, $SE = +0.6818$, -0.6439 , $n = 151$). Brood size was not influenced by foundress relatedness (multiple foundress replicates only: $F_{1,123} = 1.81$, $p = .181$, %Dev = 1.17, $n = 133$) or by its interaction with foundress number ($F_{4,122} = 0.96$, $p = .432$, %Dev = 2.48) or with host size ($F_{1,118} = 0.02$, $p = .881$, %Dev = 0.01). For groups of 1-4 foundresses, brood size increased with host size but for groups of 6 and 8 it declined (Host size: $F_{1,140} = 7.79$, $p = .006$, %Dev = 4.37, $n = 151$; Foundress number: $F_{5,144} = 4.15$, $p = .002$, %Dev = 11.64; Interaction: $F_{5,144} = 5.16$, $p < .001$, %Dev = 14.48, Figure 8a).

In terms of the number offspring produced per foundress, production was not affected by foundress relatedness (multiple foundress replicates only: $F_{1,123} = 0.41$, $p = .522$, %Dev = 0.19, $n = 133$) or by its interaction with foundress number ($F_{4,122} = 0.71$, $p = .589$, %Dev = 1.33) or with host size ($F_{1,118} = 0.30$, $p = .586$, %Dev = 0.14). For groups of 1-4 foundresses, per-foundress offspring production increased with host size and decreased with foundress number, whereas for groups of 6 and 8, production declined with host size (Host size: $F_{1,140} = 33.62$, $p < .001$, %Dev = 7.91, $n = 151$; Foundress number: $F_{5,144} = 6.44$, $p < .001$, %Dev = 7.57; Interaction: $F_{5,144} = 3.07$, $p = .012$, %Dev = 3.62, Figure 8b).

3.10 | Adult offspring size

The average (within broods) size of adult female offspring ranged between 0.6 and 1.35 mm (mean thorax length = 1.0484, $SE = \pm 0.0125$). Size was not affected by foundress relatedness (multiple foundress replicates only: $F_{1,127} = 0.19$, $p = .667$, $r^2 = .001$, $n = 133$) or by its interaction with foundress number ($F_{4,122} = 1.08$, $p = .371$, $r^2 = .03$) or with host size ($F_{1,118} = 0.07$, $p = .796$, $r^2 = .00$). Female offspring were larger when hosts were larger ($F_{1,150} = 54.19$, $p < .001$, $r^2 = .26$, $n = 151$, Figure 9a) but size was not affected by foundress number ($F_{5,149} = 1.55$, $p = .179$, $r^2 = .04$; Host size \times Foundress number interaction: $F_{5,144} = 1.02$, $p = .410$, $r^2 = .03$). Females offspring were larger when they had emerged from larger broods ($F_{1,149} = 9.73$, $p = .002$, $r^2 = .06$, $n = 151$, Figure 9b).

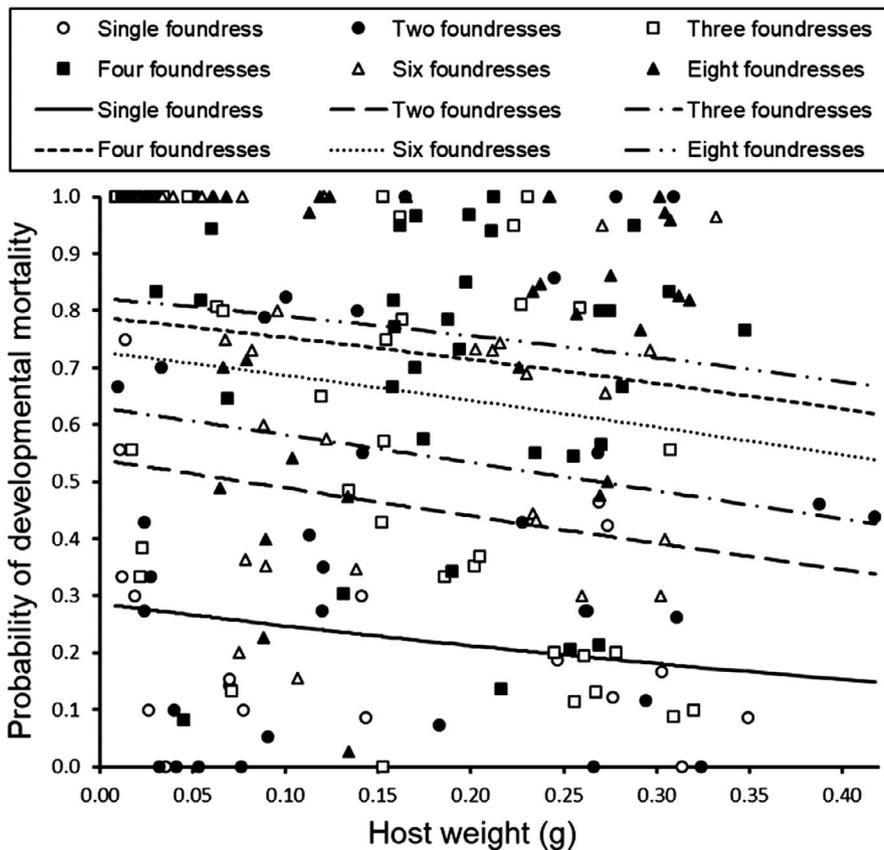


FIGURE 7 Effects of host weight and foundress number on offspring mortality

3.11 | Adult offspring sex ratio

Of the 151 replicates that produced adult offspring, 28 contained males only, most likely due to the mothers being unmated and

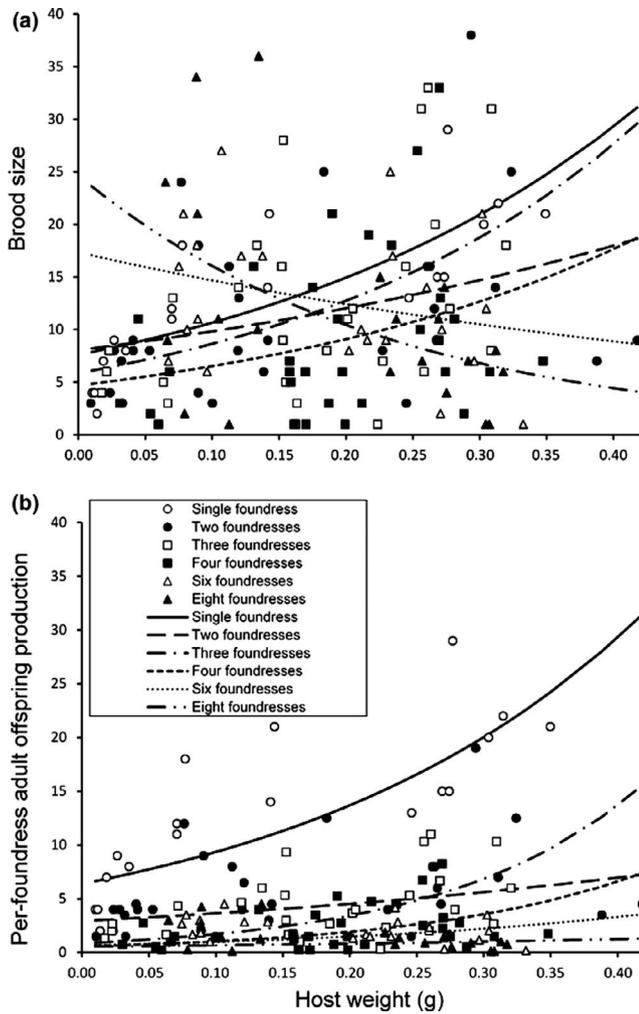


FIGURE 8 Interactive effects of foundress number and host size on the number of adult offspring produced. (a) Overall production from a given host; (b) per-foundress production

constrained to lay haploid eggs (Godfray, 1990): 15 of these replicates were single foundress or sibling foundress replicates in which insemination failure was likely due to individual single males. The remaining nonsibling foundress replicates were mostly either those in which there were small number of foundresses, making it likely that they all had been failed by a small number of different males, or replicates in which offspring production was low (e.g., eight nonsibling foundresses producing just one adult offspring), making it likely that female eggs were laid but failed to survive. Following procedures adopted by prior studies of *G. nephandidis* sex allocation (Hardy & Cook, 1995), we excluded these all-male broods, leaving 123 replicates for the analysis of brood sex ratios.

The sex ratios of groups of adult offspring (males/total offspring) were female-biased (mean proportion male = 0.2529, SE = +0.0255, -0.0240). Sex ratios of offspring produced by sibling foundresses were significantly more female-biased compared to sex ratios of offspring produced by nonsiblings (multiple foundress replicates only, $n = 108$: $F_{1,99} = 25.08$, $p < .001$, %Dev = 17.45, Figure 10). There was a significant interaction between foundress relatedness and number ($F_{4,102} = 3.85$, $p = .006$, %Dev = 10.71), such that groups of nonsiblings produced higher and relatively invariant sex ratios, whereas sibling foundresses typically produced lower sex ratios except when in the largest foundress groups. However, this interaction effect was not significant when a large outlier brood produced by eight sibling foundresses was excluded from the data ($F_{4,101} = 0.35$, $p = .556$, %Dev = 0.23). Sex ratios were significantly affected by foundress number ($F_{5,122} = 2.63$, $p = .028$, %Dev = 9.90), but not influenced by host size ($F_{1,117} = 0.10$, $p = .749$, %Dev = 0.08) or by an interaction between foundress number and host size ($F_{5,116} = 1.70$, $p = .141$, %Dev = 6.39) or between foundress relatedness and host size ($F_{1,99} = 3.82$, $p = .054$, %Dev = 2.39). Sex ratios were unaffected by the amount of resource available per foundress ($F_{1,106} = 0.02$, $p = .897$, %Dev = 0.01) nor were they affected by an interaction between relatedness and resource per foundress ($F_{1,105} = 3.08$, $p = .082$, %Dev = 2.37).

We explored sex ratio patterns among multi-foundress replicates further by analysing male and female offspring production separately. The number of males per brood was not affected by

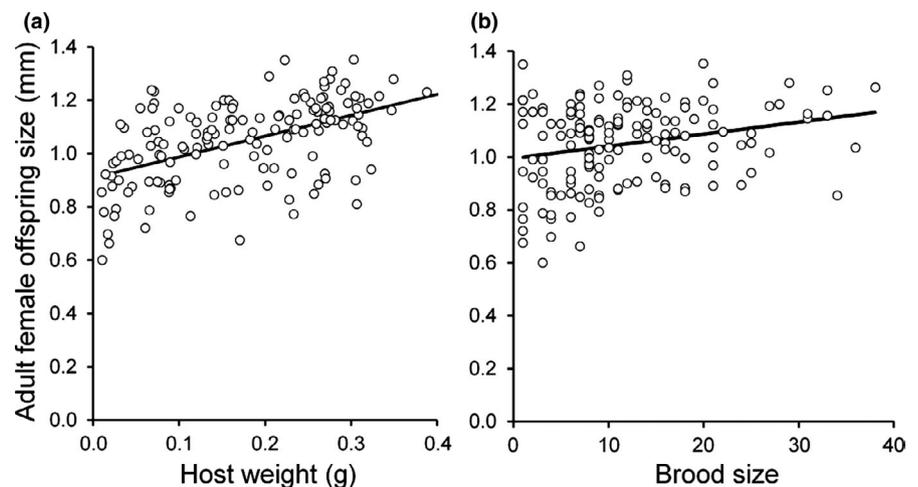


FIGURE 9 Effects of host size (a) and brood size (b) on offspring size

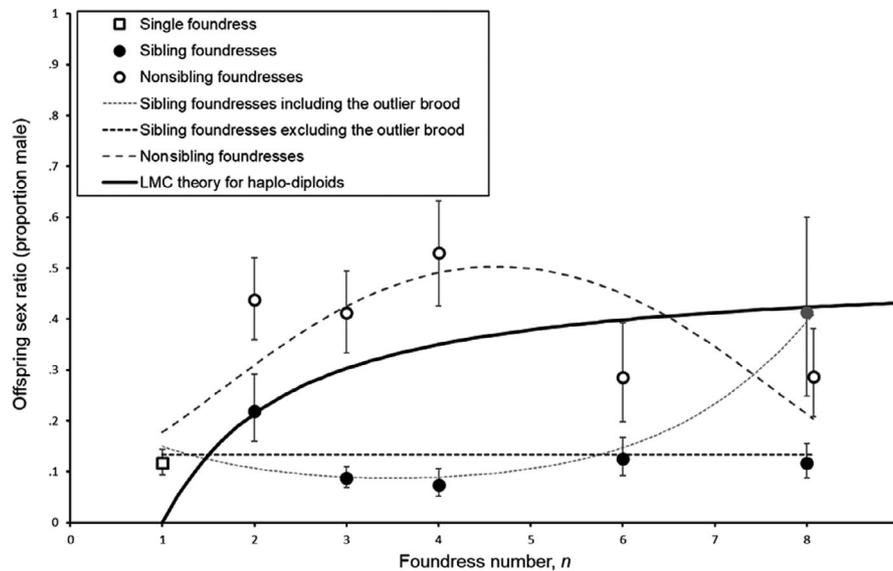


FIGURE 10 The relationship between offspring sex ratio and foundress number (n) and relatedness. Data points show means with errors bars of ± 1 SE (some are asymmetric due to the bounded nature of proportional data). Analysis of all multiple foundress replicates indicated that sex ratio was affected by relatedness and a number \times relatedness interaction (see main text). The fitted logistic regression lines illustrate the statistical minimal adequate model sex ratio response of foundresses to increasing foundress number when foundress groups are either siblings or nonsiblings; these regressions were carried out separately and included data from the single foundress case. The regression for sibling foundresses was carried out including and excluding the outlying brood (the grey symbol illustrates the mean sex ratio with this brood included). The two lower lines are the fitted logistic regression for sex ratio for sibling foundresses when data include the outlier brood [thicker line: $y = 0.1338$] and the fitted quadratic logistic regression for sex ratio among sibling foundresses when the outlier brood is excluded [the thinner line: $y = 1/(1+(1/(\exp(-0.673n)+(0.0955n^2)-1.156))))$]. The dashed line shows the fitted logistic regression for sex ratios produced by nonsibling foundresses [$y = 1/(1+(1/(\exp(1.082n)-(0.1166n^2)-2.498))))$]. Also illustrated is the predicted evolutionarily stable sex ratio according to the number of foundresses whose offspring will mate prior to dispersal, for species with haplo-diploid genetics $[(n-1)(2n-1)]/[n(4n-1)]$ (Hamilton, 1979)

foundress number ($F_{5,117} = 1.04$, $p = .397$, %Dev = 4.26, $n = 123$) but was higher when relatedness was lower ($F_{1,99} = 11.72$, $p < .001$, %Dev = 9.22, $n = 108$, Figure 11a). There was an interaction between these main effects ($F_{4,102} = 3.88$, $p = .006$, %Dev = 12.23) but this was not significant when the outlier brood for foundress number 8 was removed ($F_{4,101} = 1.10$, $p = .360$, %Dev = 3.37). There was no significant effect of host size ($F_{1,100} = 1.09$, $p = .300$, %Dev = 0.80) nor were there interactions between host size and foundress number ($F_{4,96} = 0.96$, $p = .434$, %Dev = 2.84) or relatedness ($F_{1,91} = 1.06$, $p = .306$, %Dev = 0.78). The mean number of males produced per foundress in each replicate was lower among sibling foundress groups ($F_{1,103} = 17.62$, $p < .001$, %Dev = 11.89, $n = 108$) and declined with an increase of foundress number ($F_{5,117} = 4.75$, $p < .001$, %Dev = 16.86, $n = 123$) without interaction ($F_{4,102} = 2.15$, $p = .080$, %Dev = 5.81, Figure 11c).

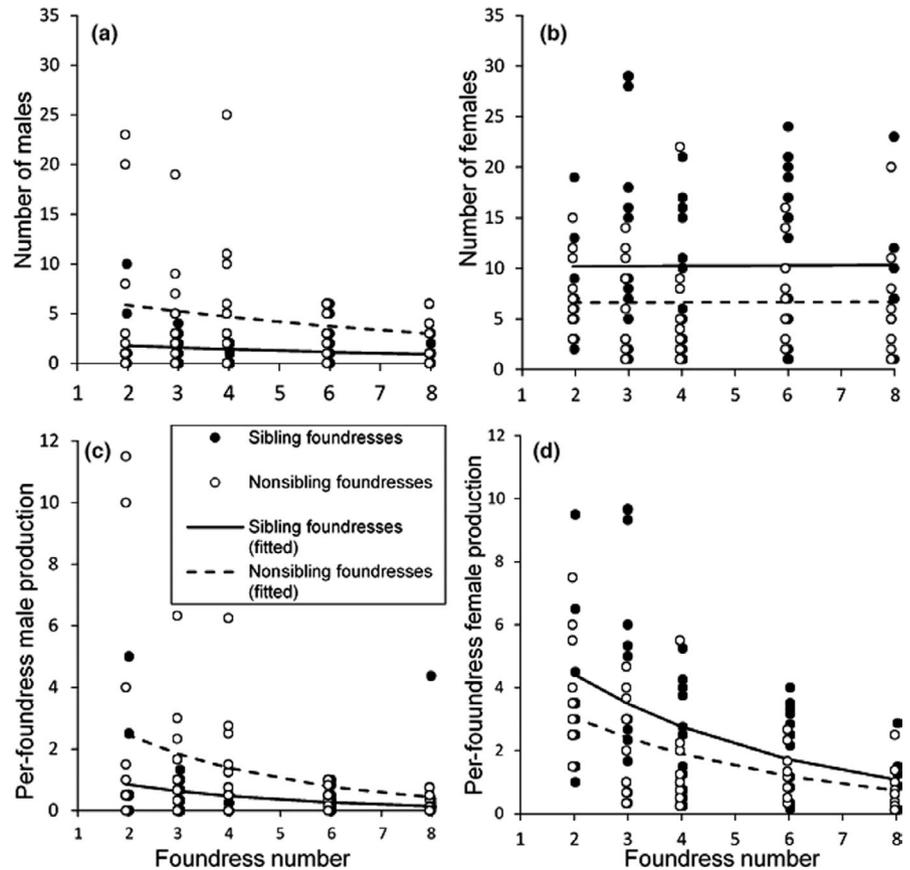
The number of females per brood was greater among broods produced by sibling foundresses than among nonsibling foundresses ($F_{1,103} = 7.38$, $p = .008$, %Dev = 6.41, $n = 108$, Figure 11b). There was no significant interaction between foundress number and relatedness ($F_{4,102} = 0.68$, $p = .611$, %Dev = 2.35) or between relatedness and host size ($F_{1,91} = 0.24$, $p = .623$, %Dev = 0.26). There was an interaction between foundress number and host size, such that for broods produced by 1–4 foundresses, female production increased with host size but for 6 and 8 foundress broods, it decreased (Interaction: $F_{5,110} = 3.40$, $p = .007$, %Dev = 11.69: Foundress

number: $F_{5,116} = 1.92$, $p = .097$, %Dev = 7.65; Host size: $F_{1,120} = 3.66$, $p = .058$, %Dev = 3.05, $n = 123$). Mean female production per foundress was greater among sibling foundress groups ($F_{1,103} = 6.56$, $p = .012$, %Dev = 4.37, $n = 108$) and declined with foundress number ($F_{5,117} = 32.33$, $p < .001$, %Dev = 58.01, $n = 123$), without significant interaction between numbers and relatedness ($F_{4,102} = 1.10$, $p = .362$, %Dev = 2.93, Figure 11d).

4 | DISCUSSION

Effects of kinship on parasitoid life histories have been relatively little explored, while there is a contrastingly substantial literature on effects of host size and of foundress number (Charnov, Los-den Hartogh, Jones, & van den Assem, 1981; Godfray, 1994; Heinz & Parrella, 1989; Ode & Hardy, 2008; Salt, 1938; Tang et al., 2014; West, 2009; Zaviezo & Mills, 2000). Considering kinship between *G. legneri* females, Lizé et al. (2012) found that aggression during short-term contests for paralysed hosts was reduced when females were recognized as siblings. Despite being aggressive, short-term contests between females of *Goniozus* species, including *G. nephantidis*, are very rarely fatal (Hardy et al., 2013; Humphries et al., 2006). Our current results, however, suggest that interactions over longer-time scales can lead to the more common death of females (see also Venkatesan et al., 2009). Some of the observed foundress mortality

FIGURE 11 Effects of foundress number and relatedness on the sexual composition of multi-foundress broods



may have been caused by host death or other factors, but the effect of relatedness is likely to be due to direct agonistic interactions or, indirectly, to females being excluded from access to the host (and thus haemolymph feeding). We found that foundress mortality only occurred when resources were the most limiting (i.e. when hosts were small and/or when foundress numbers were large). This is in accord with prior studies on *G. nephantidis* showing that both contest outcomes and aggression within contests are correlated with the value of the contested resource (Humphries et al., 2006; Stockermans & Hardy, 2013). Current evidence thus indicates that, in line with our initial expectations, the intensity of intraspecific competition in *G. nephantidis* depends simultaneously on both kinship and resource availability (West, Pen, & Griffin, 2002, provide a general review of the associated theory); in some other Hymenopteran systems, one or the other of these selective factors appears to dominate (Giron et al., 2004; West, Murray, Machado, Griffin, & Herre, 2001).

Host exploitation by multiple foundress is generally considered disadvantageous to individual mothers because it increases resource competition among their progeny (van Alphen & Visser, 1990; Dorn & Beckage, 2007; Godfray, 1994; Harvey, Poelman, & Tanaka, 2013). Under natural conditions, *G. nephantidis* is not thought to share host resources with conspecifics but using experimental conditions of enforced close contact (Legner & Warkentin, 1988; Venkatesan et al., 2009) shows that it is within the behavioural repertoire of females to tolerate the presence of conspecifics on a host (see also Sreenivas & Hardy, 2016; M. Velasco Hernández & I.C.W. Hardy, unpublished data). Beyond competition for access to resources, we

found further effects of kinship on the probability and timing of reproduction and the sexual composition of broods on maturity.

Foundress number and relatedness combined to influence the probability of reproduction, such that when foundress groups were large, oviposition was more likely when foundresses were siblings. The time taken to start laying eggs was also less when foundresses were siblings and (similar to findings for other bethylids, Gao et al., 2016) when there were more foundresses. Once oviposition commenced, we found, as with prior studies of *G. nephantidis* (Hardy et al., 1992; Humphries et al., 2006), that single foundresses laid larger clutches on larger hosts. Further, we presented females with a larger range of host sizes and observed clutch size maxima around twice the previously reported value (33 vs. 18 eggs; Hardy et al., 1992). We also found that larger groups of foundresses produced larger clutches than single foundresses, although the average per-foundress contribution of eggs was reduced, in line with initial expectations (see also Venkatesan et al., 2009). Females may have chosen to reduce the number of eggs they laid in anticipation of future competition to be experienced by their offspring (Goubault et al., 2007; Mesterton-Gibbons & Hardy, 2004; Petersen & Hardy, 1996) or their intended oviposition may have been disturbed by other females (Sreenivas & Hardy, 2016) or their deposited eggs may have been eaten by other foundresses before being experimentally observed (Goubault et al., 2007; Hardy & Blackburn, 1991; Hardy et al., 1999; Venkatesan et al., 2009): all of these are manifestations of increased competition when multiple foundresses are present.

The finding that groups of sibling foundresses ultimately produced similarly sized groups of offspring to those produced by non-sibling groups is not consistent with the notion that the observed forms of competition and interference are reduced by close kinship. Although kinship may promote the evolution of cooperative reproduction in some parasitoids and other hymenopterans (Abbot et al., 2011; Abdi, Hardy, et al., 2020; Abdi, Lupi, et al., 2020), in *G. nephantidis* there appears to be little advantage for individual females to reproduce in multi-foundress groups, as single foundresses had the highest per capita production of adult offspring, once a host was paralysed. Similarly, individual *G. nephantidis* females were able to suppress even the largest hosts they were presented with and, as such, they are unlikely to gain substantial direct fitness benefits from communal host attack in the manner reported for quasi-social species of *Sclerodermus* (Tang et al., 2014). Given that female *G. nephantidis* are mutually aggressive but tolerate other foundresses most when they are kin or when hosts are large, they may be bordering on a degree of sociality that (facultatively) includes a shared breeding site, somewhat similar to species of *Melittobia* (Hym.: Eulophidae) (Matthews & Deyrup, 2007; Matthews, Gonzalez, Matthews, & Deyrup, 2009). Present evidence suggests that *Melittobia*, *Copidosoma* and *Sclerodermus* are the most socially advanced genera of parasitoids; all of them typically attack hosts which are large relative to the size of the adult wasps and effects of kinship have also been reported (Abdi, Hardy, et al., 2020; Abdi, Lupi, et al., 2020; Giron et al., 2004; Giron & Strand, 2004) or suggested (Matthews et al., 2009) in these genera. We consider that if there are *Goniozus* species that naturally attack very large hosts, these are the most likely members of the genus to exhibit more socially advanced behaviour than *G. nephantidis*.

The sex ratios of broods produced by individual *G. nephantidis* females are typically female-biased (around 9% of offspring are males; Hardy & Cook, 1995; Hardy et al., 1998; Hardy et al., 1999) and largely conform to expectations from the classical LMC theory for the single foundress case (Godfray, 1994; Hamilton, 1967, 1979; West, 2009). Effects of foundress number and relatedness have not previously been explored (except in the context of optimizing foundress number for mass-rearing efficiency, Venkatesan et al., 2009, where relatedness was not considered), largely because multiple foundress clutches are not thought to occur in nature. Nonetheless, there was a sex ratio response to an increase in foundress number among non-sibling groups that broadly followed our expectation from classical LMC theory attuned to the haplo-diploid genetics of hymenopterans (Hamilton, 1979) and is also similar to responses observed in many other parasitoid and nonparasitoid species (Burton-Chellew et al., 2008; Godfray, 1994; Hu et al., 2010; Ode & Hardy, 2008; Werren, 1983). Current evidence for *G. nephantidis* does not, thus, fit with the notion that natural selection for adaptations to uncommon situations might be too weak to lead to facultative sex ratio adjustment (Burton-Chellew et al., 2008; Herre, 1985; Innocent, Savage, West, & Reece, 2007; Shuker, Reece, Taylor, & West, 2004; Shuker, Reece, Whitehorn, & West, 2004; West, 2009). We note that nonfacultative sex allocation can also generate sex ratios that

conform to LMC predictions but this relies on male eggs being oviposited early into clutches, followed by female eggs (Chung, Pienaar, & Greeff, 2019; Zhang, Dunn, & Wang, 2020): current evidence for sex allocation sequences for *Goniozus* suggests that males are laid at the end of the oviposition bout (Khidir et al., 2012).

In contrast to sex ratios produced by nonsibling foundresses, the sex ratios of broods produced by groups of siblings tended to be similar to those produced by single foundresses, irrespective of foundress number. Classical LMC theory implicitly assumes that the sexual compositions of offspring groups at oviposition and at maturity are equivalent, whereas this will not be the case when there is developmental mortality which alters the sexual composition of brood prior to the time of mating (Green et al., 1982; Hardy & Cook, 1995; Hardy et al., 1998; Khidr et al., 2012; Nagelkerke & Hardy, 1994; Wilkinson, Kapranas, & Hardy, 2016). We found that mortality became substantially more common as foundress numbers increased, thus reducing our ability to evaluate correspondence between observed and predicted sex ratios. Nonetheless, as we detected no difference in developmental mortality between broods produced by siblings and nonsiblings, mortality patterns are not a likely cause of the observed effect of kinship on the sex ratios of offspring that developed successfully: we thus consider that the observed difference is most likely due to sex allocation responses by ovipositing foundresses.

Sex ratio theory has developed from Hamilton's original models to consider further the influence of relatedness; a typical result is that increased interactions between kin (sibmating or closely related foundress females) select for greater female bias (Burton-Chellew et al., 2008; Frank, 1985; Hu et al., 2010; Innocent et al., 2007; Shuker, Reece, Whitehorn, et al., 2004; Taylor & Crespi, 1994; West, 2009). The patterns we observe among *G. nephantidis* broods are thus consistent with predictions of models considering co-foundress relatedness (and we note that sex ratios of the congener *G. legneri* respond to the relatedness between a female and her mate in the predicted direction, K. Du, M.K. Abdi, I.C.W. Hardy, unpublished data). There have been very few evaluations of the sex ratio response of parasitoids to foundress relatedness, or indeed among other species, and prior evidence has not collectively supported theory (Burton-Chellew et al., 2008; Shuker, Reece, Taylor, et al., 2004). Studies by Shuker, Reece, Taylor, et al. (2004) and Burton-Chellew et al. (2008), for instance, found that *Nasonia vitripennis* co-foundresses do not adjust their offspring sex ratio according to whether they are sisters or nonrelatives. They considered that *N. vitripennis* may not be able to assess their relatedness to other foundresses in the vicinity, possibly because they so rarely oviposit on the same patch as close relatives that there will be limited selection for kin discrimination and thus facultative sex ratio adjustment. In contrast, although the known biology of *G. nephantidis* also suggests that multi-foundress offspring group production will be rare in nature, kin recognition between adult female *Goniozus* has been demonstrated (Lizé et al., 2012), most likely based on variation in cuticular hydrocarbons (Khidir, Linforth, & Hardy, 2013), and co-foundresses apparently adjust sex allocation according to kinship in the direction that theory predicts.

5 | CONCLUSIONS

The potential for the evolution of sociality among bethylids, and what this might imply about hymenopteran sociality more generally, has been of long-standing interest (Evans, 1978; Gorokhovskaya, 2015; Malyshev, 1968; Wheeler, 1928). The present study was designed to explore whether prolonged female–female contact would elicit behavioural and reproductive responses that might reveal incipient higher-level sociality in a naturally sub-social species, *G. nephantidis*. We conclude that under many circumstances, females will not be selected to share the resources of a paralysed host with other females. However, selection against sharing appears to be less stringent when either relatedness or resource availability is higher, in line with initial expectations. Further, despite being presented with that is thought to be an unnatural reproductive environment, females in multi-foundress groups apparently attune sex allocation according to both foundress number and foundress relatedness. *Goniozus nephantidis* appears to be the only studied parasitoid in which sex ratios conform to predictions of both classical and extended local mate competition theories.

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CONFLICT OF INTEREST

All authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

All authors designed the experiment. M.K.A. collected the data. M.K.A. and I.C.W.H. analysed the data and wrote the manuscript. I.C.W.H. revised the manuscript. All authors discussed the work at all stages and approved the manuscript.

DATA AVAILABILITY STATEMENT

The data generated in the experiments reported in this study are available from the corresponding author on reasonable request and from the Dryad data repository (<https://doi.org/10.5061/dryad.905qftth8>).

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