

Kinship effects in quasi-social parasitoids I: co-foundress number and relatedness affect suppression of dangerous hosts

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Explanations for the highest levels of sociality typically invoke the concept of inclusive fitness. *Sclerodermus*, a genus of parasitoid hymenopterans, is quasi-social, exhibiting cooperative brood care without generational overlap or apparent division of labour. Foundress females successfully co-exploit hosts that are too large to suppress when acting alone and the direct fitness benefits of collective action may explain their cooperation, irrespective of kinship. However, cooperation in animal societies is seldom free of conflicts of interest between social partners, especially when their relatedness, and thus their degree of shared evolutionary interests, is low. We screened components of the life-history of *Sclerodermus brevicornis* for effects of varying co-foundress number and relatedness on cooperative reproduction. We found that the time taken to paralyse standard-sized hosts is shorter when co-foundress number and/or relatedness is higher. This suggests that, while females must access a paralysed host in order to reproduce, individuals are reluctant to take the risk of host attack unless the benefits will be shared with their kin. We used Hamilton's rule and prior data from studies that experimentally varied the sizes of hosts presented to congeners to explore how the greater risks and greater benefits of attacking larger hosts could combine with relatedness to determine the sizes of hosts that individuals are selected to attack as a public good. From this, we predict that host size and relatedness will interact to affect the timing of host paralysis; we test this prediction in the accompanying study.

ADDITIONAL KEYWORDS: cooperation – Hamilton's rule – kinship – *Sclerodermus brevicornis* – sociality.

INTRODUCTION

Understanding the evolution and maintenance of complex social systems has been a long-standing and major focus of behavioural and life-history research across a diverse array of animal taxa (Wilson, 1971; Choe & Crespi, 1997; Costa, 2006; Bourke, 2011; Scheiber *et al.*, 2017; Shen *et al.*, 2017; Boomsma & Gawne, 2018; Tanaka *et al.*, 2018). A substantial portion of this sociobiological research has been carried out on eusocial hymenopterans, such as ants, honeybees, bumble bees and vespine wasps, all of which exhibit brood care, reproductive division of labour and

overlapping generations (Bourke & Franks, 1995; Crozier & Pamilo, 1996; Boomsma & Gawne, 2018). Conversely, sociality in the parasitoid Hymenoptera ('parasitic wasps') is relatively little explored, chiefly because most species are socially solitary, lacking parental care (Wilson, 1971; Godfray, 1994) and there are no known lineages of eusocial parasitoids (Peters *et al.*, 2017). However, species in the genus *Sclerodermus* (Latreille) (Hymenoptera: Aculeata: Bethyliidae) exhibit cooperative brood care, apparently without division of labour, classing them as quasi-social (Li & Sun, 2011; Liu *et al.*, 2011; Tang *et al.*, 2014; Wang *et al.*, 2016; Lupi *et al.*, 2017) and are the focus of the current study.

Sclerodermus females search actively for host larvae, which they paralyse by stinging (Xu *et al.*, 2002; Shao-chuan, 2007; Yao & Yang, 2008; Liu *et al.*, 2011).

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A host may be attacked by several females (Bridwell, 1920; Kühne & Becker, 1974; Tang *et al.*, 2014; Lupi *et al.*, 2017). The females (termed foundresses) then oviposit onto the host's integument and subsequently tend, cooperatively, their collective broods of offspring as these feed on the host, until and beyond their pupation (Hu *et al.*, 2012; Wu *et al.*, 2014). Quasi-social behaviour in *Sclerodermus* has been explained in terms of the direct fitness benefits to cooperation accrued by individuals within groups of females that attack hosts: females acting alone may not be able to suppress and successfully reproduce on a host, especially if the host is large and dangerous to attack (Kühne & Becker, 1974; Liu *et al.*, 2011; Tang *et al.*, 2014; Wei *et al.*, 2014), a 'collective action benefit' (Shen *et al.*, 2017). This explanation does not, however, preclude that kin selection effects, based on the genetic relatedness between interacting individuals (inclusive fitness; Hamilton, 1964), may also influence the occurrence and success of cooperative reproduction and thus the evolution of quasi-sociality. Despite ongoing controversy, inclusive fitness is often invoked to understand the evolution of a range of social behaviours, including conflict resolution, cooperation and eusociality (Abbot *et al.*, 2011; Nowak & Allen, 2015; Marshall, 2016; Birch, 2017; Shen *et al.*, 2017). Among the parasitoid Hymenoptera it has been shown that aggression during resource competition, whether between larvae or between adult females, can be reduced when competitors are (actual or perceived) close relatives (Giron *et al.*, 2004; Giron & Strand, 2004; Lizé *et al.*, 2012; Dunn *et al.*, 2014; Mathiron *et al.*, 2019). Hence, we expected that *Sclerodermus* co-foundresses may adjust their cooperative behaviour according to intra-group relatedness.

Our overarching aim is to explore the importance of kinship effects among parasitoid hymenopterans that have evolved quasi-sociality. Using *S. brevicornis*, we evaluate whether relatedness between co-foundresses influences their reproductive performance, in terms of suppressing hosts, reproducing on suppressed hosts, foundress mortality, and the characteristics of any offspring subsequently produced. We screen for effects of relatedness at numerous life-history stages because conflicts of interest between co-foundresses may be manifest at any, all or none of these. For instance, foundresses may act as non-attacking free-loaders (Rankin *et al.*, 2007) exploiting the host attack efforts of others, they may have differing interests in terms of their relative contributions to the production and care of offspring groups post-host paralysis, and differing sex ratio optima (Tang *et al.*, 2014). As our investigation is in consequence relatively 'untargeted' (*sensu* Snart *et al.*, 2015) we also test our key conclusions in a more targeted accompanying study (Abdi *et al.*, 2020a).

The advantages to an individual foundress of sharing a host of a given size may depend on the number of co-foundresses with which it is shared (Tang *et al.*, 2014); we thus explore relatedness effects when there are either two or four foundresses, using standardized hosts of relatively small size. Although intra-group genetic relatedness is manipulated in our core experiment, we further assess behaviours when co-foundresses have been reared on the same or on different host species and also according to whether foundresses are winged or wingless. Host-of-origin effects may be expected if foundresses use cuticular hydrocarbon cues to assess relatedness (Supporting Information, Appendix S1) and wing dimorphism effects may be expected if alate females tend to disperse further and co-found broods with non-relatives whereas apterous females tend to co-found with prior brood-mates (Supporting Information, Appendix S2).

We find evidence for greater production of offspring per foundress when foundresses are prior brood-mates than when they are non-siblings and also that the time taken to paralyse hosts is shorter when inter-foundress relatedness is higher. We then use a simple public goods model (Hamilton's rule) to illustrate how co-foundress relatedness and the greater risks and benefits of attacking larger hosts could combine to determine the size of host that a female is selected to attack to provide resources shared with co-foundresses. From this, we predict that host size and relatedness will interact to affect the timing of host paralysis. The accompanying study (Abdi *et al.*, 2020a) tests this prediction empirically and supports our conclusions.

MATERIALS AND METHODS

INSECTS

Sclerodermus brevicornis (Kieffer) (Hymenoptera: Bethyilidae) is a gregarious ectoparasitoid wasp. Adult females are typically wingless (apterous); however, winged (alate) morphs also occur (Lupi *et al.*, 2017). Males are usually alate; however, apterous morphs are occasionally produced. *S. brevicornis* is a parasitoid of European longhorn beetle larvae (Coleoptera: Cerambycidae) (Lupi *et al.*, 2017) that can be reared on several non-native, invasive cerambycids, including the yellow longhorn beetle, *Psacotheta hilaris hilaris* (Pascoe) (Lupi *et al.*, 2017) and also on larvae of the rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) (Abdi *et al.*, 2020b): these two species were used as hosts in this study. Stocks of *P. h. hilaris* were maintained according to Lupi *et al.* (2015) and *C. cephalonica* were maintained in the same climate chamber, on the standard diet described by Limonta *et al.* (2009). Stocks of *S. brevicornis* were reared on late instar larvae of *P. h. hilaris* and *C. cephalonica*.

All cultures and experiments were maintained in a climate chamber at 25 ± 1 °C, and 16 h: 8 h L: D photoperiod and $60 \pm 5\%$ relative humidity. To synchronize parasitoid and host availability, newly emerged *S. brevicornis* adults were stored in a refrigerator at 5 ± 0.5 °C for a maximum of 7–10 days prior to use in experiments (such storage does not affect their subsequent performance; D. Lupi & C. Jucker, unpublished data, based on several hundred replicates).

EXPERIMENT

The experiment followed a core 2×2 factorial design, varying foundress number and relatedness, but with additional consideration of wing dimorphism and of the host species on which foundresses had been reared (Table 1). There were 20 replicates of each combination of foundress number, relatedness, wing dimorphism and host species, giving 160 replicates in total (Table 1).

In each replicate, a *C. cephalonica* host was placed into a 10 mL glass vial (10 cm long \times 1.3 cm diameter). Hosts were selected to be of near uniform size (mean weight = 0.0363 mg, range = 0.0262 to 0.0534 mg, SD \pm 0.0051: note that the mean head capsule width of *C. cephalonica* hosts in this size range is c. 1.02 mm, corresponding to the ‘small’ host size class of *P. h. hilaris*; Lupi *et al.*, 2017). Then either two or four adult female *S. brevicornis* were added into each vial.

In some replicates (Table 1, rows A & C) co-foundresses had developed in the same brood: as these broods had been produced by several (in most cases sibling) mothers, brood-mates were a mix of siblings and (typically closely related) non-siblings that are likely to recognize each other as familiar (Lizé *et al.*, 2012). In all other replicates, co-foundresses had developed in separate broods and were non-siblings (Table 1). In some non-sibling replicates, foundresses had developed on (separate) *C. cephalonica* hosts and in other non-sibling replicates (Table 1, rows E & H) half the foundresses present (one or two) had developed on a *C. cephalonica* host and other half had developed on *P. h. hilaris*. In most replicates all foundresses were apterous; however, in one set of non-sibling replicates (Table 1, row G) the foundresses were alate and had developed on *P. h. hilaris*. The allocation of individual wasps to treatments could not be entirely random, because some were allocated as sibling pairs and others as individuals; the pattern of allocation was as haphazard as possible within the constraints of the biological material available. Within replicates, we size-matched females by eye and discarded any unusually small females from the experiment.

The vials were closed with a gauze and cotton wool, left undisturbed for 4 days (observing the wasps

within this period has previously been found to disrupt their reproductive behaviours, D. Lupi & C. Jucker, pers. obsv.). We then observed each replicate once per day until any offspring matured to the pupal stage (typically 28 days after foundresses were presented with hosts). At each observation, we recorded whether the host had been paralysed (paralysed hosts no longer made major movements with their bodies and usually small brown dots were visible at the site of venom injection), the number of eggs laid on the host, whether any foundresses had died (until the twelfth day in all core replicates, tenth day in host species-of-origin replicates and until the eighth day in wing dimorphism replicates) and the developmental stage of any juvenile offspring after eclosion from eggs. We further monitored broods for up to 42 days in total, noting the timing of adult emergence. The numbers, sexes, wing morphologies and size (thorax length) of adult offspring were recorded on their emergence from the pupal cocoons.

STATISTICAL ANALYSES

The explanatory variables foundress number and relatedness (main effects) were fitted as factors, and their interaction was included in initial statistical models. As foundress number in a replicate could change during the experiment due to foundress mortality, we explored the effects of both initial and current foundress number in some analyses. Effects of host origin and wing morphology, fitted as factors, are reported in the Supporting Information. The response variables were the parameters recorded during the daily observations and at the end of the experiment. All statistical tests were two sided. Unless otherwise stated, significance was assessed by sequentially deleting terms from initially complex models, and aggregating levels within factors, to achieve minimal adequate models (Crawley, 1993; Wilson & Hardy, 2002). All analyses were carried out using the statistical software package *GenStat* (v.17, VSN International, Hemel Hempstead, UK).

Binary data on host acceptance and offspring production were explored using logistic analyses Generalized Linear Model (GLM) assuming binomially distributed errors and the reported test statistic is change in deviance, G , which is approximately χ^2 distributed (Crawley, 1993). Other logistic analyses of proportional data with denominators larger than unity (e.g. probability of paralysis and oviposition, developmental mortality, offspring sex ratio) assumed quasi-binomial error distributions and the reported test statistic is the F -ratio (Crawley, 1993; Wilson & Hardy, 2002). Logistic analyses comparing foundress mortality before and after host paralysis employed mixed models [Generalized Linear Mixed Model (GLMM) and Wald χ^2

Table 1. Experimental design and rationale

Rows A, B, C and D constitute the core 2 × 2 factorial experiment, designed to evaluate effects of foundress number, foundress relatedness and their interaction, with host species-of-origin and foundress wing morphology held constant. Results of analyses focusing on these replicates are reported in the main body of this paper and summarized in [Table 2](#).
 Replicates in further rows were included to provide additional tests. Data from rows B, E and F allow evaluation of effects of host-species-of-origin (fitted as a factor with three levels) for the 2-foundress case, as cuticular hydrocarbon profiles of foundresses are likely to be affected by their developmental host and may be used in kinship assessment. Comparison of data from rows B and F served also to check for intrinsic effects of developing on a beetle larva rather than on a moth larva: given that *Sclerodermus* species are not reported to attack lepidopteran hosts in nature, such differences might imply that host-of-origin cannot be regarded as a simple proxy for relatedness. Data from rows B, D, E, and H provide a 2 × 2 analysis of foundress number and host-of-origin effects within non-sibling groups. Results of analyses focusing on host-of-origin effects are reported in the Supporting Information ([Appendix S1](#)).
 Comparison of data from rows F and G provide tests of effects of foundress wing morphology on the reproductive strategies for the 2-foundress case. In nature, alate females may be expected to disperse as individuals and thus to typically reproduce with unrelated co-foundresses, whereas apterous females may tend to disperse together with their brood-mates. Alate female *S. brevicornis* are relatively rare; a shortage of biological material precluded us from including further replicates with winged foundresses. Results of analyses of the effects of wing dimorphism are reported in the Supporting Information ([Appendix S2](#)).

Row	Number of foundresses	Foundress relatedness	Host of foundress origin	Foundress morphology	Replicates (total = 160)
Core replicates					
A	Two	Brood-mates	<i>C. cephalonica</i>	Apterous	20
B	Two	Non-siblings	<i>C. cephalonica</i>	Apterous	20
C	Four	Brood-mates	<i>C. cephalonica</i>	Apterous	20
D	Four	Non-siblings	<i>C. cephalonica</i>	Apterous	20
Additional replicates (Supporting Information, Appendices S1-S2)					
E	Two	Non-siblings	<i>C. cephalonica</i> + <i>P. h. hilaris</i>	Apterous	20
F	Two	Non-siblings	<i>P. h. hilaris</i>	Apterous	20
G	Two	Non-siblings	<i>P. h. hilaris</i>	Alate	20
H	Four	Non-siblings	<i>C. cephalonica</i> + <i>P. h. hilaris</i>	Apterous	20

statistics], with replicate fitted as a random factor. All logistic analyses adopted a logit-link function.

Integer data (e.g. clutch size, number of offspring produced) were explored using log-linear analyses assuming quasi-Poisson error distributions and a log-link function (Crawley, 1993). Data derived from integers (e.g. offspring production per foundress) that were likely to be approximately Poisson-distributed were also analysed using log-linear models (Faraway, 2006; Tang *et al.*, 2014). 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, oviposition, adult offspring emergence and foundress mortality were analysed using parametric cohort survival analyses with censoring (Aitkin *et al.*, 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). In cohort-survival analyses of factors potentially influencing time-to-foundress death, the longevity of each foundress was treated as if it were independent of others in the replicate. Such pseudo-replication is likely to generate Type I errors (false significance) but as none of our analyses detected significant effects we adopt these interpretations.

As we performed many individual statistical tests, we controlled for possible Type I errors using the false discovery rate (FDR) procedure to control for multiple comparisons (Benjamini & Hochberg, 1995). We treated results from the core experiment as a 'family' of tests (including evaluations of interactions (Cramer *et al.* 2016)) and set the family-wide α -value to 0.25. We adopted this relatively high false discovery rate due to the exploratory nature of our study (McDonald, 2014) and the tendency of multiple comparisons corrections to generate Type II errors. We report uncorrected initial interpretations in the main text and any adjustments to these following FDR procedures in the results summary tables (Table 2 and Supporting Information; Appendix S1, Table A1.1, Appendix S2, Table A2.1 and Data set S1).

RESULTS

Results of analyses reported in this section are summarized in Table 2.

FOUNDRESS MORTALITY

Around a quarter (26.67%, 64/240) of foundresses died by the twelfth day after presentation with hosts. Mortality

across this 12-day period was not affected by foundress relatedness (logistic ANOVA: $G_1 = 1.17$, $P = 0.282$, %Dev = 1.51), foundress number ($G_1 = 0.37$, $P = 0.543$, %Dev = 0.48) or their interaction ($G_1 = 0.30$, $P = 0.584$, %Dev = 0.39). Within this 12-day period, the timing of foundress mortality (Fig. 1) was not significantly affected by foundress relatedness, foundress number or their interaction (cohort survival analysis: Relatedness: $G_1 = 2.5$, $P = 0.114$, %Dev = 0.83; Number: $G_1 = 0.8$, $P = 0.371$, %Dev = 0.31; Interaction: $G_1 = 0.7$, $P = 0.403$, %Dev = 0.24). In these analyses, foundresses that did not die by the twelfth day were treated as censors.

We re-examined these data by separately assessing the proportion and timing of foundress mortality during the periods before host paralysis and after host paralysis. For all replicates combined, the probability of at least one member of the group of foundresses dying was significantly higher during the pre-paralysis period than post-paralysis (GLMM: Wald $\chi^2 = 20.08$, d.f. = 1, $P < 0.001$). Similarly, across all replicates, the proportion of foundress that died pre-paralysis was significantly higher than the proportion that died post-paralysis (GLMM: Wald $\chi^2 = 19.22$, d.f. = 1, $P < 0.001$, Fig. 2). Analysing the data separately for each combination of foundress number and relatedness found that proportions of foundresses dying were significantly different before and after paralysis for both of the 2-foundress cases (2-brood-mates: Wald $\chi^2 = 17738.16$, d.f. = 1, $P < 0.001$; 2-non-siblings: Wald $\chi^2 = 1537597.45$, d.f. = 1, $P < 0.001$); indeed none of these foundresses died post-paralysis, but not for the 4-foundress cases, in which some foundresses died post-paralysis (4-brood-mates: Wald $\chi^2 = 19.8$, d.f. = 1, $P = 0.089$; 4-non-siblings: Wald $\chi^2 = 1.99$, d.f. = 1, $P = 0.167$).

In terms of the timing of mortality, prior to host paralysis, foundresses in groups of two died marginally non-significantly earlier than those in groups of four (cohort survival analysis: $G_1 = 3.8$, $P = 0.0513$, %Dev = 1.43). Mortality rate was not significantly affected by relatedness ($G_1 = 2.3$, $P = 0.1294$, %Dev = 0.86) or by the interaction between foundress relatedness and number ($G_1 = 0.6$, $P = 0.439$, %Dev = 0.26). In this analysis, foundresses that did not die by the day of host paralysis were treated as censors. After host paralysis, foundresses in groups of four tended to die significantly earlier than foundresses in groups of two ($G_1 = 6.7$, $P = 0.0096$, %Dev = 9.34), as no post-paralysis mortality occurred in 2-foundress groups. Time to death was not affected by foundress relatedness ($G_1 = 0.2$, $P = 0.6547$, %Dev = 0.31) or by an interaction between foundress relatedness and number ($G_1 = 0.00$, $P = 1.00$, %Dev = 0.00). In these analyses, we included all foundresses that were alive at the time of host paralysis and those that did not die by the twelfth day were treated as censors.

Table 2. Summarized core experiment results*

Reproductive trait	Relatedness	Foundress number	Interaction
Foundress mortality (proportion)	NS†	NS	NS
Foundress mortality (timing) before host paralysis	NS	Marginal significance: foundresses in small groups die earlier	NS
Foundress mortality (timing) after host paralysis	NS	Foundresses in large groups die earlier	NS
Paralysis of host (proportion)	NS	Large groups paralyse more hosts	NS
Time to paralysis of host	Brood-mates paralyse the host earlier§	Large groups paralyse the host earlier	NS
Oviposition on host (probability)	NS	NS	NS
Time from paralysis to oviposition on host	Marginal significance: non-siblings oviposit earlier¥	NS	NS
Clutch size [CS] (total)	NS	NS	NS
Per-foundress CS (using initial foundress number [FN])	NS	Smaller in large groups	NS
Per-foundress CS (using FN at oviposition)	NS	Smaller in large groups	NS
Offspring developmental mortality	NS	NS	NS
Reproductive success (probability)	NS	Greater in large groups	NS
Brood size [BS]	NS	NS	NS
Per-foundress BS (using initial FN)	Marginal significance: brood-mates produce more offspring¥	Smaller in large groups	NS
Per-foundress BS (using FN at oviposition)	Marginal significance: brood-mates produce more offspring	Smaller in large groups	NS
Brood developmental period (using initial FN)	Significance via interaction	Significance via interaction	Slower development when small groups are brood-mates, and when large groups are non-siblings
Adult offspring size (using FN at oviposition)	Non-siblings produce larger offspring	Small groups produce larger offspring	NS
Sex ratio of offspring	NS	NS	NS

* Significant effects with no footnote retained the same interpretation after multiple-comparisons correction using a false discovery rate of 0.25.

† NS = No statistically significant influence detected in the initial analysis.

§ Evidence for earlier host attack by more closely related foundresses also found in the accompanying study (Abdi *et al.*, 2020a).

¥ Non-significant after multiple-comparisons correction.

PARALYSIS

The percentage of hosts that were paralysed by *S. brevicornis* foundresses was 78.75% ($N = 80$). This proportion was not affected by co-foundress relatedness (logistic ANOVA: $G_1 = 0.85$, $P = 0.355$, %Dev = 1.03) but was greater in 4-foundress replicates (97.5%) than in 2-foundress replicates (60%) ($G_1 = 19.57$, $P < 0.001$, %Dev = 23.64). There was no significant interaction

between foundress relatedness and number ($G_1 = 0.97$, $P = 0.324$, %Dev = 1.18).

Among the 63 replicates in which host paralysis occurred, the time taken to paralyse the host ranged up to 10 days. The 17 replicates in which there was no paralysis on the twelfth day were treated as censors in the subsequent analysis of influences on the time taken to paralyse hosts. Time-to-paralysis was dependent on both relatedness and foundress number

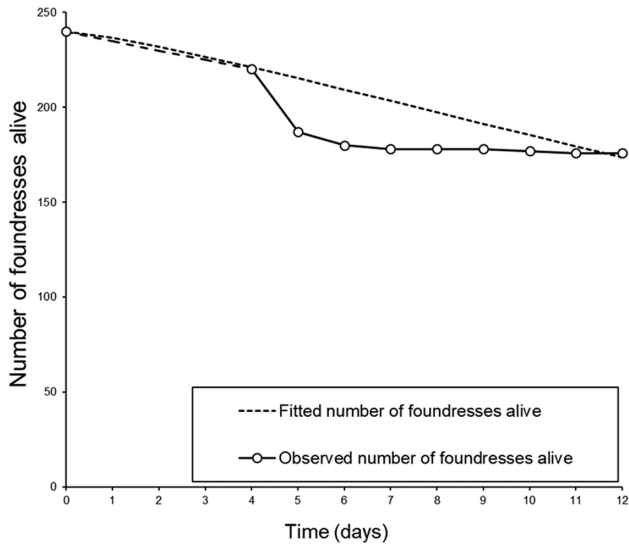


Figure 1. Cohort survival of *Sclerodermus* females. The fitted line (short dashes) was estimated using Weibull analysis for time-dependent hazard rates (rate parameter = 0.01451, shape parameter = 1.25) with foundresses that survived until day 12 treated as censors. To avoid disruption of the females' behaviours, no observations were taken on days 1, 2 and 3, indicated by the longer-dashed line, and the statistical model was fitted assuming no foundresses died during this period.

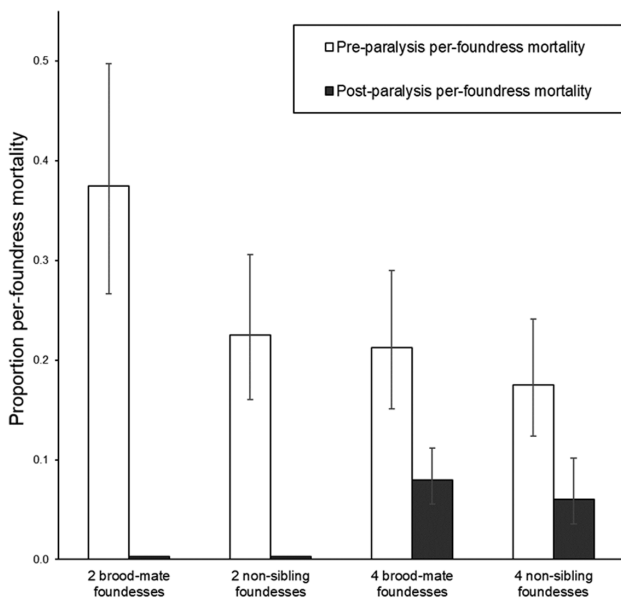


Figure 2. Proportion of foundresses dying before and after host paralysis. The overall proportion of per-foundress mortality in the period before host paralysis (white bars) was significantly higher than in the period after paralysis (dark gray bars) (see main text). Standard errors of means are asymmetrical due to back transformation from the logit scale.

but not on their interaction (cohort survival analysis: Relatedness: $G_1 = 4.0$, $P = 0.0455$, %Dev = 3.63; Number: $G_1 = 24.0$, $P < 0.0001$, %Dev = 18.44; Interaction: $G_1 = 0.2$, $P = 0.6547$, %Dev = 0.29), with hosts being paralysed more rapidly when there were four foundresses present and when foundresses were brood-mates (Fig. 3): generally, 4-foundress groups paralysed hosts faster than 2-foundress groups while the paralysis in both types of groups was more rapid when co-foundresses were brood-mates.

OVIPOSITION

The percentage of paralysed hosts which were oviposited on was 92.06% ($N = 63$). The probability of oviposition was not significantly affected by foundress relatedness (logistic ANOVA: $G_1 = 0.15$, $P = 0.701$, %Dev = 0.42), number ($G_1 = 0.82$, $P = 0.365$, %Dev = 2.35) or their interaction ($G_1 = 1.12$, $P = 0.29$, %Dev = 3.2). The time from host paralysis to the start of egg laying ranged up to 8 days and was not significantly affected by foundress relatedness (cohort survival analysis: $G_1 = 3.2$, $P = 0.0736$, %Dev = 3.8), number ($G_1 = 0.1$, $P = 0.7518$, %Dev = 0.07) or their interaction ($G_1 = 0.2$, $P = 0.655$, %Dev = 0.28).

CLUTCH SIZE

Clutches laid by foundress groups ranged between three and 33 eggs ($N = 58$, mean = 16.24, SE = +0.83, -0.79) and did not vary according to foundresses relatedness (log-linear ANOVA: $F_{1,56} = 0.38$, $P = 0.54$, %Dev = 0.70), initial foundress number ($F_{1,57} = 0.02$, $P = 0.887$, %Dev = 0.04) or their interaction ($F_{1,55} = 0.14$, $P = 0.707$, %Dev = 0.26). Although *C. cephalonica* hosts were selected to be of near uniform size (see above), we checked for an influence of host weight on clutch size: there was no significant relationship ($F_{1,56} = 0.98$, $P = 0.327$, %Dev = 1.72).

The average number of eggs produced per initial foundress (clutch size divided by the initial number of foundresses, for the 58 replicates in which oviposition occurred) was not affected by foundresses relatedness (log-linear ANCOVA: $F_{1,56} = 0.56$, $P = 0.457$, %Dev = 0.54) but was significantly smaller in 4-foundress replicates than in 2-foundress replicates ($F_{1,57} = 48.26$, $P < 0.001$, %Dev = 46.84). Per-foundress egg production was not affected by an interaction between foundress relatedness and number ($F_{1,55} = 0.15$, $P = 0.704$, %Dev = 0.14). Taking into account that, due to foundress mortality, in some replicates foundress number at the time of oviposition was lower than the initial number, we similarly found that the number of eggs per foundress was unaffected by relatedness (log-linear ANCOVA: $F_{1,54} = 0.37$, $P = 0.543$, %Dev = 0.28) and was significantly lower when foundress numbers

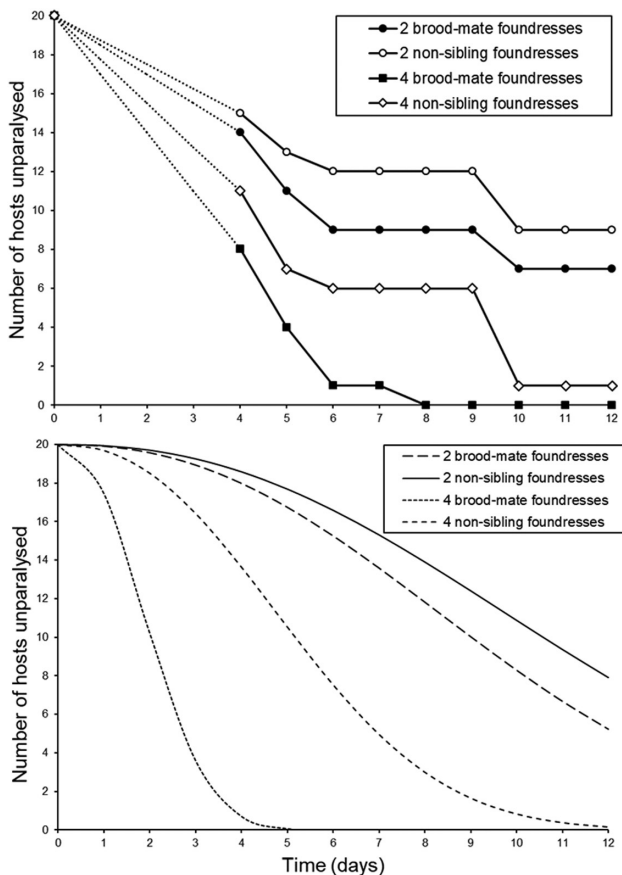


Figure 3. The effects of co-foundress relatedness and number on time to paralyse the host. Hosts were paralysed earlier when foundresses were related and when there were more foundresses. The upper panel shows the cohort data from the four treatments and the lower panel shows the fitted survival model, estimated from Weibull analysis with unparalysed hosts treated as censors. To avoid disruption of the females' behaviours no observations were taken on days 1, 2 and 3, and the statistical model was fitted assuming no hosts were paralysed during this period.

were greater ($F_{3,57} = 27.81$, $P < 0.001$, %Dev = 61.72, Fig. 4), with no interaction between relatedness and the number alive at the time of oviposition ($F_{3,53} = 0.45$, $P = 0.715$, %Dev = 1.01).

OFFSPRING DEVELOPMENTAL MORTALITY

The percentage of eggs that failed to develop to adulthood was 14.97% ($N = 58$ clutches). Egg-to-adult mortality was not affected by foundress relatedness (logistic ANOVA: $G_1 = 0.71$, $P = 0.402$, %Dev = 1.24), foundress number ($G_1 = 2.48$, $P = 0.121$, %Dev = 4.3) or their interaction ($G_1 = 0.47$, $P = 0.496$, %Dev = 0.82).

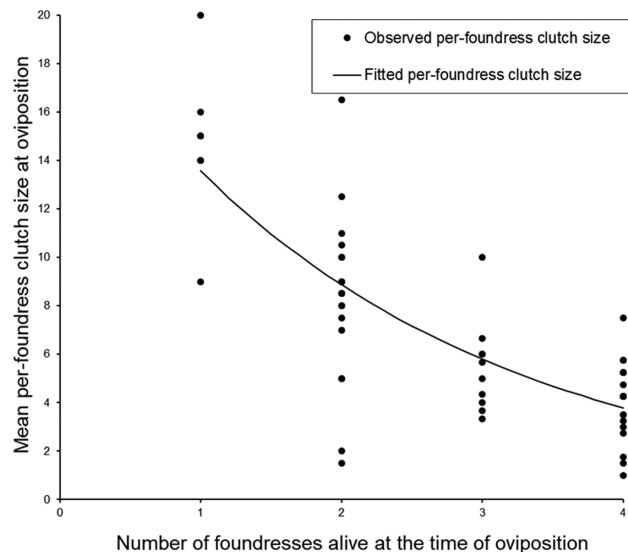


Figure 4. The effect of foundress number at the time of egg laying on per-foundress clutch size at oviposition. The mean number of eggs produced per foundress was lower when more foundresses were alive at the time of oviposition.

OFFSPRING PRODUCTION

The probability that at least some adult offspring were produced from a given host (i.e. reproductive success, defined as a binary response, for all provided hosts, $N = 80$) was 0.66. This probability was unaffected by foundresses relatedness (logistic ANCOVA: $G_1 = 0.28$, $P = 0.595$, %Dev = 0.30) but was significantly greater among 4-foundress replicates (mean = 0.875) than among 2-foundress replicates (mean = 0.575) ($G_1 = 9.42$, $P = 0.002$, %Dev = 10.01). There was no interaction between foundress relatedness and number ($G_1 = 0.05$, $P = 0.824$, %Dev = 0.05).

BROOD SIZE

The total number of adult offspring produced from a host which had been oviposited on ($N = 53$), ranged between 3 and 32 (mean = 15.11, SE = +0.71, -0.67). Brood size was not affected by foundresses relatedness (log-linear ANCOVA: $F_{1,51} = 2.56$, $P = 0.116$, %Dev = 4.84), initial foundress number ($F_{1,52} = 1.26$, $P = 0.266$, %Dev = 2.39) or by their interaction ($F_{1,50} = 0.11$, $P = 0.738$, %Dev = 0.21%).

In terms of the number offspring produced per foundress, production was significantly lower when there were initially four foundresses than when there were two (log-linear ANOVA: $F_{1,52} = 82.37$, $P < 0.001$, %Dev = 60.85). The effect of foundress relatedness (per foundress offspring production was higher in sibling groups) was non-significant ($F_{1,51} = 3.07$, $P = 0.086$, %Dev = 2.27, $N = 53$); but note the relatively low P -value

and that with log-linear analyses estimated P -values are approximate rather than exact (Crawley, 1993).

There was no significant interaction between foundress relatedness and initial number ($F_{1,50} = 0.12$, $P = 0.730$, %Dev = 0.09%). Taking foundress mortality into account, we similarly found that the per-foundress production of offspring (brood size divided by the number of surviving foundresses at oviposition) was lower when there were more foundresses alive at the time of oviposition ($F_{1,52} = 119.74$, $P < 0.001$, %Dev = 69.37) and that there was a marginally non-significant effect of relatedness, with brood-mate foundresses producing more offspring than non-sibling foundresses ($F_{1,51} = 3.74$, $P = 0.059$, %Dev = 2.17, Fig. 5). There was no significant interaction between foundress relatedness and number at oviposition ($F_{1,50} = 0.13$, $P = 0.724$, %Dev = 0.07).

BROOD DEVELOPMENTAL PERIOD

The time from oviposition to the emergence of adult offspring was affected by foundresses relatedness and initial number via a significant interaction (cohort survival analysis of time to first emergence from each brood: Relatedness: $G_1 = 2.0$, $P = 0.157$, %Dev = 2.5; Number: $G_1 = 0.5$, $P = 0.479$, %Dev = 0.55; Interaction: $G_1 = 9$, $P < 0.01$, %Dev = 0.56): offspring developed most rapidly in 4-foundress replicates and the direction of influence of foundress relatedness was dependent on foundress

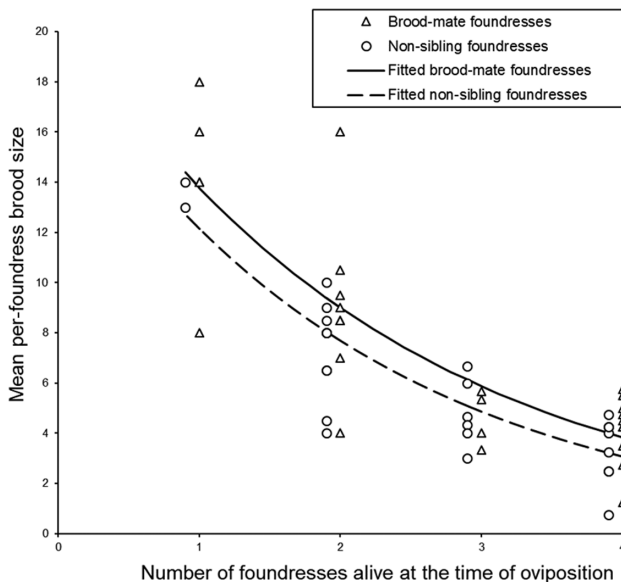


Figure 5. The effects of foundress number and relatedness on brood size produced per-foundress alive at the time of oviposition. For a given number of foundresses, broods were larger when foundresses were brood-mates. Data points are slightly laterally displaced to reduce overlap.

number (Fig. 6): for 2-foundress broods, development was most rapid when foundresses were non-siblings whereas for 4-foundress broods, non-sibling foundresses produced slower developing offspring. Taking into account that in some replicates not all initial foundresses survived until eggs were laid, we similarly found that developmental time was affected by an interaction between relatedness and the number of foundresses alive at the time of oviposition ($G_1 = 10.9$, $P < 0.001$, %Dev = 17.11).

ADULT OFFSPRING SIZE

The average thoracic size of adult female offspring ranged between 0.58 and 0.91 mm (mean = 0.764, SE \pm 0.011). The mean (per brood) size of female offspring was marginally non-significantly (positively) influenced by increasing per-offspring resource availability (host weight divided by the number of egg laid onto the host) (standard regression: $F_{1,51} = 3.53$, $P = 0.066$, $r^2 = 0.065$, $N = 53$ broods). Adults produced by non-sibling foundresses were significantly larger than those of brood-mate foundresses ($F_{1,50} = 9.12$, $P = 0.004$, $r^2 = 0.14$, Fig. 7) and body size was also negatively affected by the number of foundresses alive at the time of oviposition ($F_{1,50} = 4.27$, $P = 0.045$, $r^2 = 0.066$, Fig. 7). There was no significant interaction between foundress number and relatedness ($F_{1,50} = 3.25$, $P = 0.078$, $r^2 = 0.052$).

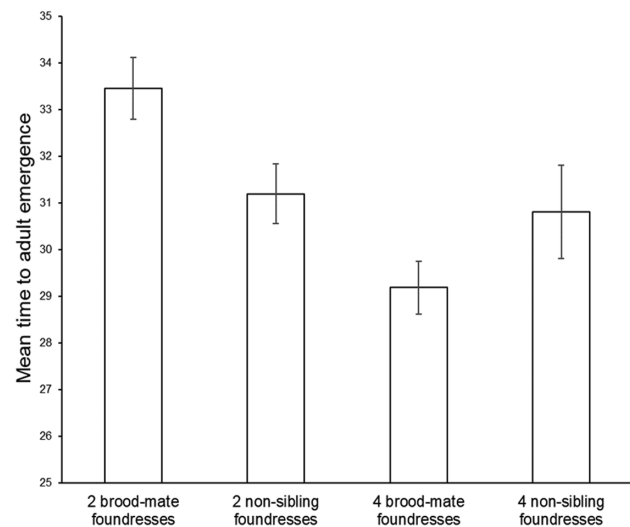


Figure 6. Developmental time of offspring (days). Among broods produced by two foundresses, offspring developed faster when the foundresses were not brood mates whereas among broods produced by four foundresses the opposite pattern was observed.

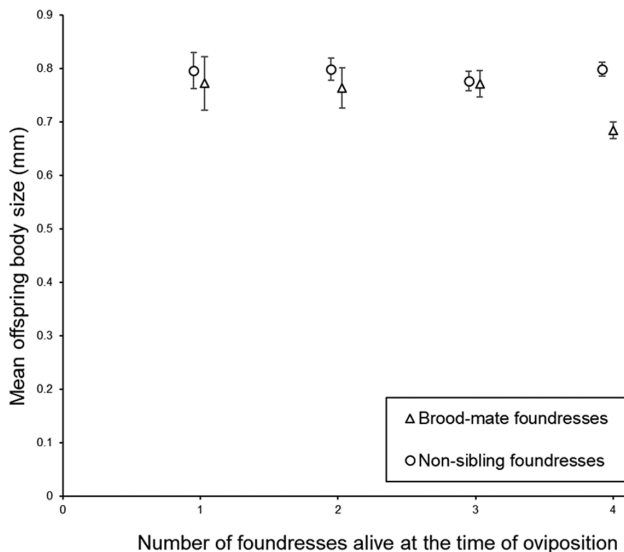


Figure 7. Influences on offspring body size. The size (thorax length) of adult female offspring was influenced by both relatedness and the number of foundresses alive at the time of oviposition. There was also a marginally non-significant influence of resource per developing offspring, such that offspring were larger when resources were less limited. Data for non-siblings are slightly laterally displaced to reduce overlap.

SEX RATIO

Sex ratios of adult offspring groups were highly female-biased (mean proportion male = 0.0787, SE = +0.0075, -0.0069, $N = 53$) and were under-dispersed (Heterogeneity Factor = 0.571). Sex ratios were not significantly influenced by foundresses relatedness (logistic ANOVA: $G_1 = 0.06$, $P = 0.813$, %Dev = 0.11, $N = 53$), initial foundresses number ($G_1 = 0.30$, $P = 0.585$, %Dev = 0.60) or their interaction ($G_1 = 1.08$, $P = 0.305$, %Dev = 2.13). Taking foundress mortality into account, we similarly found that sex ratios were unaffected by the number of foundresses alive at the time of oviposition or by foundress relatedness (Number: $G_1 = 1.34$, $P = 0.253$, %Dev = 2.49; Relatedness: $G_1 = 0.05$, $P = 0.824$, %Dev = 0.09; Interaction: $G_1 = 3.34$, $P = 0.074$, %Dev = 6.22). Sex ratios were also not significantly affected by brood size ($F_{1,51} = 0.08$, $P = 0.781$, %Dev = 0.0015) or by the percentage of developmental mortality experienced by the brood ($F_{1,51} = 1.10$, $P = 0.299$, %Dev = 0.021).

Offspring groups that contained no adult males at emergence were not uncommon (mean proportion of all-female broods = 0.1698, SE = +0.0557, -0.0442). The probability that an offspring group was all-female declined significantly with increase in brood size ($G_1 = 53.60$, $P < 0.001$, %Dev = 51.24) and increased significantly with the prevalence of developmental mortality within the brood ($G_1 = 15.18$, $P < 0.001$,

%Dev = 22.93). Neither foundress number nor relatedness had any significant effects on these relationships. The data gathered are available as supporting information (Supporting Information, Data set S1).

DISCUSSION

This study evaluated potential effects of co-foundress number and relatedness throughout the offspring-production cycle of the quasi-social parasitoid *S. brevicornis*. Variation in *Sclerodermus* foundress number has been experimentally evaluated before (Tang *et al.*, 2014; Gao *et al.*, 2016b; Wang *et al.*, 2016; Wei *et al.*, 2017; Abdi *et al.*, 2020b), whereas variation in foundress relatedness has not. For foundress number, we found that groups of four foundresses were more successful, and also more rapid, in paralysing hosts than were pairs of foundresses. Hosts of *Sclerodermus* are typically large and dangerous to attack, with several prior studies reporting that hosts can kill the attacking female (Kühne & Becker, 1974; Liu *et al.*, 2011; Wei *et al.*, 2014), and our data additionally show that foundress mortality is typically higher pre-host paralysis than post-paralysis. It is unsurprising that larger groups of females perform better in host attack as this result has been found in several prior studies on *Sclerodermus* species (Li & Sun, 2011; Tang *et al.*, 2014; Gao *et al.*, 2016b; Abdi *et al.*, 2020b). There are several potential mechanisms for the results: the probability of the host eventually becoming paralysed may equal the sum of independent probabilities of individual attack successes, or attacks may become successively more likely to succeed as the dose of venom injected into the host increases, or foundresses may coordinate their attacks as a team. Although larger *S. brevicornis* foundress groups were more successful in terms of attacking hosts, similar numbers of offspring were produced from hosts paralysed by two or four females and thus the per-foundress reproductive output was lower. Tang *et al.* (2014) have previously shown that the costs and benefits to *Sclerodermus* females of sharing a host with other foundresses are host-size dependent, essentially mediated by the lower probability of success for individual or small groups of foundresses when faced with larger, and likely more dangerous, hosts (Liu *et al.*, 2011; Wei *et al.*, 2017).

For foundress relatedness, we found that many aspects of performance were unaffected by whether foundresses were brood-mates (which will often be siblings) or non-siblings (originating from different broods). Similarly, Wu *et al.* (2017) found that foundress-offspring relatedness had negligible effects on offspring development in *Sclerodermus guani*. However, we found that groups of brood-mate foundresses paralysed

hosts sooner than did non-sibling groups and also that, once hosts were suppressed, there was a (marginally significant) greater production of offspring per foundress when foundresses were prior brood-mates than when they were non-siblings. Greater numbers of offspring developing on a host of a given size led to adults produced by brood-mates having smaller body size, similar to results for *Sclerodermus harmandi* (Liu *et al.*, 2011) and other bethylids (Mayhew, 1998) and small body size may constitute a fitness disadvantage (Gao *et al.*, 2016a).

Tang *et al.* (2014) concluded that *Sclerodermus* quasi-sociality (multi-foundress cooperation in brood production) could be explained in terms of direct fitness benefits accruing to females when hosts are too large to be likely to be suppressed by an individual foundress, but added that effects of kinship between foundresses may also be found to operate. The shorter latency to host paralysis observed among groups of prior brood-mates indicates that foundresses are more prone to take the risk of attacking and paralysing a host when the beneficiaries of a successful attack include their kin. We note that evidence for earlier host attack by more closely related foundresses is also found in the accompanying study (Abdi *et al.*, 2020a).

The likelihood of *Sclerodermus* co-foundresses being kin in the field is unknown. Females may disperse from their natal patch individually and form new foundress groups effectively at random or they may tend to disperse as groups of brood mates, which will typically be kin: future work will be required to discover natural patterns of *Sclerodermus* dispersal. Because most *S. brevicornis* females are wingless but a minority have wings, we expected that wing-dimorphism might affect female's reproductive decisions. We consider alate females much more likely to disperse as individuals, and thus to typically reproduce with unrelated co-foundresses, and apterous females to be more likely to disperse and interact with relatives. We, however, found no convincing effects of this dimorphism on the behavioural characters assessed, although offspring developmental timing and offspring size were affected (Supporting Information, Appendix S2). We also explored host-of-origin as a proxy for kinship, given that the cuticular hydrocarbon profiles of foundresses are likely to be affected by their developmental host and that these profiles may in turn be used in kinship assessment. Although we found effects of the developmental host on subsequent reproductive behaviours, we also found evidence for a wider set of developmental and physiological effects that confound confident interpretation in terms of kin selection (Supporting Information, Appendix S1).

Our study contributes to the growing body of evidence for the operation of kin effects in the reproductive ecology of parasitoid wasps. These have

now been found at various life-history stages, including host attack and the defence of paralysed hosts by adults, between offspring developing on a shared host resource (Giron & Strand, 2004; Giron *et al.*, 2004; Lizé *et al.*, 2012; Dunn *et al.*, 2014; Mathiron *et al.*, 2019) and some aspects of maternal care (Wu *et al.*, 2017). For *Sclerodermus*, the existence of kin-mediated host attack and effects on per-foundress offspring production suggest that co-foundresses may attune their behaviour according to inter-foundress relatedness at other life-history stages that have not yet been investigated in detail. For instance, once a dangerous host has been suppressed, the resources it represents may be open to selfish exploitation, such that some co-foundresses produce more offspring than others (reproductive skew). As foundresses may obtain indirect fitness benefits by facilitating the reproductive output of related foundresses, we might expect any such skew to be mediated by kinship.

KINSHIP AND HOST ATTACK AS A PUBLIC GOOD

Our experiment found that groups of brood-mate foundresses paralyse hosts more rapidly than non-sibling groups. The most likely explanation is that individual foundresses delay taking the risk of host attack according to their relatedness to other females present. Here we consider further a foundress's host attack decision by using the public goods framework of Hamilton's rule (Hamilton, 1964; Cooper *et al.*, 2018; Supporting Information, Appendix S3).

Hamilton's rule asserts that a trait will spread if its fitness benefits to the recipients (b) multiplied by the relatedness (r) between social partners is greater than the fitness cost (c) to the actor ($rb - c > 0$). Public goods models explore the evolution of cooperation and consider situations in which a focal individual produces a costly public good, the benefits of which are then shared between the individual and its social partners. Inclusive fitness considerations (mediated by relatedness, r) can explain why individuals may be selected to carry out costly behaviours that benefit others, as opposed to not incurring costs and possibly benefitting from public goods produced by others (Cooper *et al.*, 2018).

For *Sclerodermus*, we suggest that a paralysed host represents a public good with the fitness benefits (b), in terms of offspring production, shared by all foundresses present. We also suggest that the fitness cost (c) to a female in providing this public good can be represented by the risk of mortality involved in attacking a host. In the experiment on *S. brevicornis* reported here, host size was held essentially constant but both b and c are likely to vary with host size: in other *Sclerodermus* species larger hosts can support the development of more offspring (Liu *et al.*, 2011;

Tang *et al.*, 2014) but are also more risky to attack (Liu *et al.*, 2011; Wei *et al.*, 2017). We use empirical results from these three prior studies to estimate the relationships between host size and costs and between host size and benefits (Supporting Information, Appendix S3).

We acknowledge that approaches to calculating b and c in Hamilton's rule are the subject of ongoing theoretical debate and development (Gardner *et al.*, 2011; Supporting Information, Appendix S3) and also that it is not possible to measure these parameters directly. We instead estimate proxies from separate empirical (statistical) regressions, one for the effect of host size on offspring production ($\approx b$) and the other for the effect of host size on the mortality risk to an attacking foundress ($\approx c$). As both are affected by host size, our proxies are in the form of regression equations rather than being fixed values applied across all host sizes, and are scaled to take values between zero and unity (Supporting Information, Appendix S3).

Using these relationships and an open formulation of Hamilton's rule, in which the mechanism generating co-foundress relatedness is undefined (Cooper *et al.*, 2018), suggests that selection for public goods host attack should depend interactively on both co-foundress relatedness, r , and host size. The maximum size of a healthy and active host that individual foundresses should be prepared to attack alone and then share, if successful, is generally larger when co-foundresses are more closely related (Fig. 8, Supporting Information (Appendix S3, Fig. A3.1)).

The simple model framework we employ heuristically is unlikely to capture exactly all of the selective forces that shape host-attack decisions in *Sclerodermus*. Further, the model functions to indicate whether a female might be expected to attack and then share a suppressed host, not whether a female should attack a host to be exploited only by her own offspring. Females that do not obtain access to a paralysed host (whether shared or as a lone foundress) during their lifetime will have zero fitness and, as such, might be expected to attack even the most dangerous hosts when other options are unavailable (analogous to the 'Desperado Effect'; Grafen, 1987).

The kinship effect that we have found empirically does not operate in terms of the eventual probability of host paralysis but in the timing of that suppression. We thus expect females encountering a dangerous host, and with limited alternative reproductive opportunities, to be selected to at first wait for other females to attack the host but eventually to attack it themselves, with reluctance to attack being reduced by inclusive fitness benefits when other females present are kin. We suggest that there is scope to usefully develop formal theory that considers how a female's attack decisions may change over time as well as how

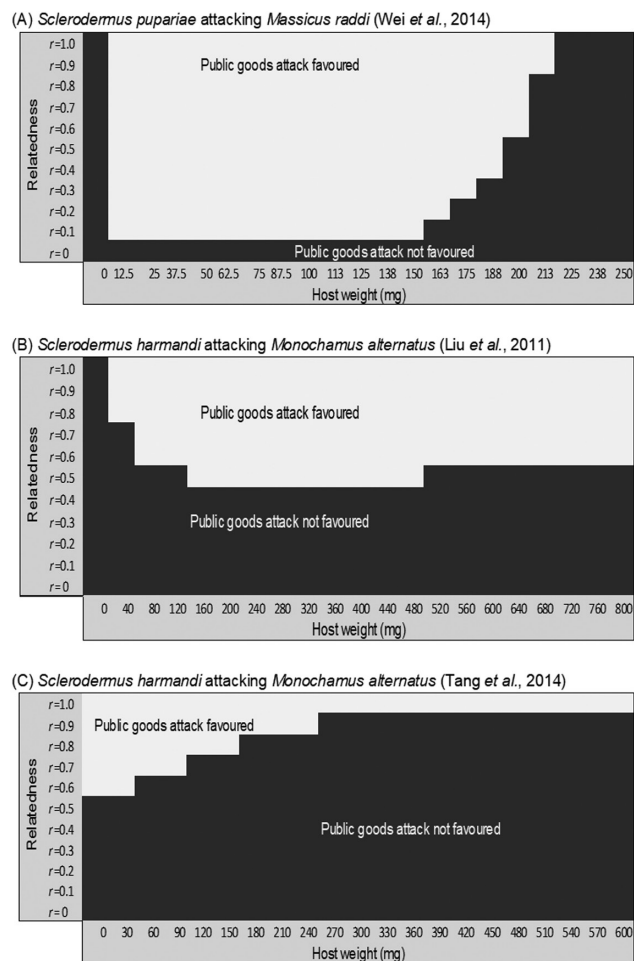


Figure 8. A public goods model of relatedness-mediated host attack. The panels illustrate how variation in relatedness (r) and in host weight combine to influence selection for public goods attack. Host weight affects the probability of a foundress dying prior to host suppression (cost, c) and also the fitness benefits obtained from paralysed hosts (benefit, b). Light areas indicate combinations that satisfy Hamilton's rule ($rb - c > 0$). These illustrations derive from three separate empirical studies, of two different *Sclerodermus*-host species associations that assessed different ranges of host sizes (panels A, B and C). Details on data sources and the relationships between host size and the cost and benefit parameters are provided in Supporting Information (Appendix S3).

attacks by individual females, whether separate or coordinated, may interact in terms of the probability of host suppression. Our expectation is that latency to attack a given host will be shorter when co-foundresses are more closely related and when the effects of individual attacks are non-independent. Currently, using Hamilton's rule to consider relatedness-based host attack in combination with cost and benefit

estimates derived from prior studies has generated the testable prediction that the time to host attack will be affected interactively by host size and co-foundress relatedness.

CONCLUSION

This study was designed to identify potential roles of foundress kinship in a quasi-social reproductive system that has hitherto been explained in terms of direct fitness advantages to co-founding broods on large hosts and without recourse to inclusive fitness considerations. We find that inter-foundress relatedness influences the timing of host attack by *S. brevicornis* females: groups of brood-mate females paralyse hosts earlier than similar-sized groups of non-sibling foundresses. A simplistic use of a public goods model framework appears to capture the essence of a female's decision to attack (and, if successful, subsequently share) a host according to her relatedness to co-foundresses but further information on the natural life-histories of *Sclerodermus* species will be required to assess and develop its key assumptions. In our experiment, host size was held constant but the public goods model framework suggests that both host size and inter-foundress kinship will interactively influence host attack behaviour. We test this assertion in the accompanying empirical study (Abdi *et al.*, 2020a).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

- Appendix S1.** Effects of foundress number and host species-of-origin.
- Appendix S2.** Effects of *Sclerodermus* females' wing dimorphism.
- Appendix S3.** Public goods models of host attack.
- Data set S1.** Data from core and additional replicates.

SUPPLEMENTARY INFORMATION FOR:

**Kinship effects in quasi-social parasitoids I: Co-foundress number
and relatedness affect suppression of dangerous hosts**

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APPENDIX 1:

EFFECTS OF FOUNDRRESS NUMBER AND HOST SPECIES-OF-ORIGIN

The mechanistic basis of parasitoid kin recognition may operate via the chemical composition of the adult exoskeleton, which may be influenced by an individual's genetics and/or by its developmental environment (Ode *et al.*, 1995; Lizé *et al.*, 2012; Khidr *et al.*, 2013). In *Sclerodermus* (B. Stockermans & I.C.W. Hardy, unpublished data), and in other bethylids (Khidr *et al.*, 2013), the profile of cuticular hydrocarbons in the adult integument is affected by the species of host on which the individual developed. For an assessor, a conspecific perceived to have developed on a different host, especially on a different species of host, is unlikely to be as closely related as a conspecific perceived to have developed on the same individual host. Hence, we expected that *Sclerodermus* foundresses may assess propinquity of relatedness to co-foundresses according to their host-of-origin and modify their cooperative reproductive behaviour accordingly.

A1.1. INTRINSIC EFFECTS OF DEVELOPING ON A BEETLE LARVA OR MOTH LARVA

Using only data from replicates containing two non-sibling foundresses (Table 1 in the main paper, rows B and F, n=40), we first checked for effects of foundress development on different host species in the absence of variation in foundress number or relatedness; such differences might indicate that host-of-origin is not a simple proxy for the assessment of relatedness. We found no significant effects on any of the life-history stages examined (paralysis, clutch size, foundress mortality, reproductive success, developmental mortality, brood size, time to adult emergence, adult offspring size, sex ratio) except for time to oviposition. The time taken for foundresses to oviposit on the hosts was significantly shorter when foundresses had been reared on *P. h. hilaris* than when they had been reared on *C. cephalonica* (cohort survival analysis: $G_1 = 19.14$, $P < 0.001$, %Dev=51.04, Fig. A1.1). We interpret this to indicate that *S. brevicornis* females that have developed on a beetle host may have an intrinsically enhanced metabolic profile (e.g. allowing them to mature eggs more rapidly), compared to those reared on a factitious

lepidopteran host. More broadly, this result indicates that further effects of host-of-origin (in the analyses using a wider set of data below) may not be the result of kinship assessment alone.

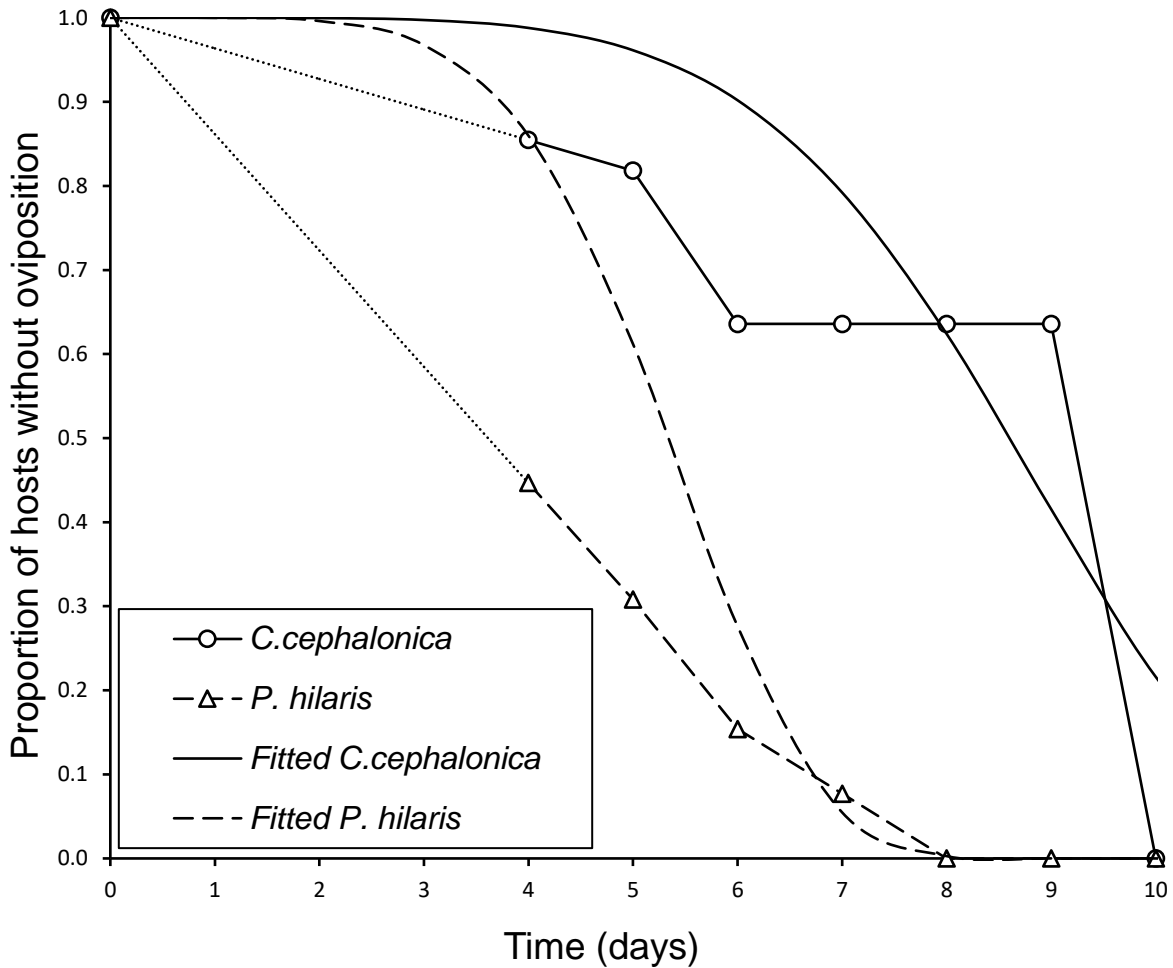


Figure A1.1. The effect of host species-of-origin on time taken to oviposition by two non-sibling foundresses. *Corcyra cephalonica* hosts were oviposited on more rapidly when foundresses were reared on beetle larvae.

Further analyses of host-of-origin effects on the behaviour and performance of unrelated co-foundresses used data from rows B, E and F (Table 1 of the main paper) (with rearing host species fitted as a factor with 3 levels) for the 2-foundress case only, and from rows B, D, E and H (with rearing host species fitted as a factor with 2-levels) for both 2 and 4 foundress cases (Table A1.1).

A1.2. FOUNDRRESS MORTALITY

Foundress mortality (proportion) was not affected by host species-of-origin (logistic ANOVA: 3-level factor: $G_2=1.38$, $P=0.261$, %Dev=4.61, $n=60$; 2-level factor: $G_1=1.66$, $P=0.202$, %Dev=2.08, $n=80$) or by an interaction with foundress number ($G_1=0.00$, $P=1.00$, %Dev=0.00). Time to foundress mortality was not significantly affected by rearing host species-of-origin (cohort survival analysis: 3-level factor: $G_2=5$, $P=0.082$, %Dev=3.64; 2-level factor: $G_1=2.9$, $P=0.089$, %Dev=1.2) or by an interaction with foundress number ($G_1=0.1$, $P=0.752$, %Dev=0.04).

A1.3. PARALYSIS

The proportion of hosts paralysed was not affected by the host species on which foundresses developed (logistic ANOVA: 3-level factor: $G_2=0.65$, $P=0.522$, %Dev=1.67; 2-level factor: $G_1=1.56$, $P=0.212$, %Dev=1.95) or by an interaction with foundress number ($G_1=0.82$, $P=0.366$, %Dev=1.02). Time to paralysis was significantly affected by rearing host species (cohort survival analysis: 2-level factor: $G_1=12.9$, $P<0.001$, %Dev=16.42) and by foundress number ($G_1=22.5$, $P<0.001$, %Dev=21.75), with hosts being most rapidly paralysed when there were four co-foundresses that had developed on a mix of host species (Fig. A1.2). There was, however, no significant interaction with foundress number ($G_1=0.7$, $P=0.403$, %Dev=1.95). There was no significant effect when host-of-origin was treated as a factor with three levels ($G_2=3.3$, $P=0.192$, %Dev=3.49, $n=60$).

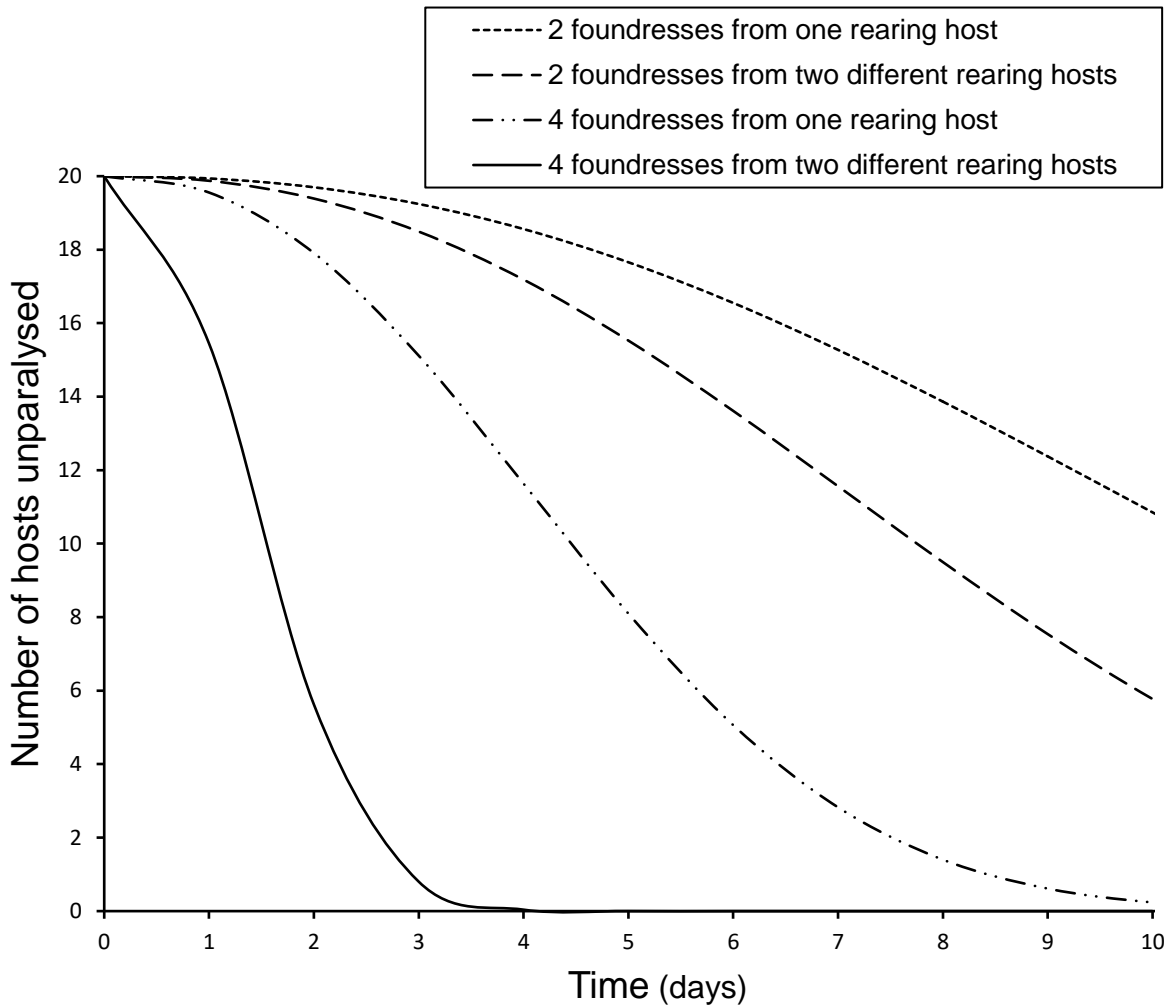


Figure A1.2. The effect of host species-of-origin on time taken to paralyse hosts. *Corcyra cephalonica* hosts were paralysed more rapidly when foundresses had been reared on a mixture of host species, and when there were more foundresses. The figure shows the fitted cohort survival curves from Weibull analysis, with hosts unparalysed on the 10th day treated as censors.

A1.4. OVIPOSITION

The probability of oviposition was significantly affected by host species-of-origin, such that co-foundresses that had been reared from two different host species were more likely to lay eggs than those had been reared on *C. cephalonica* (logistic ANCOVA: 2-level factor: $G_1=4.85$, $P=0.028$, %Dev=16.20, $n=64$). There was a significant interaction effect between foundress number and host species-of-origin, with oviposition probability being higher when foundress numbers were greater and co-foundresses had been reared on different host species, but lower when co-foundresses were reared in *C. cephalonica*

($G_1=5.42$, $P=0.020$, %Dev=18.12). The probability of oviposition was not affected by host species-of-origin treated as a factor with three levels ($G_2=1.23$, $P=0.292$, %Dev=11.65, $n=39$).

In terms of time to oviposition, when host species-of-origin was treated as a 2-level factor, there was a significant interaction between foundress number and host species (cohort survival analysis: Number: $G_1= 1.00$, $P=0.3173$, %Dev=1.18; Host-of-origin: $G_1= 7.00$, $P<0.01$, %Dev=9.21; Interaction: $G_1= 4.2$, $P<0.05$, %Dev=5.97), with eggs being laid earliest when there were four co-foundresses that had been reared on different host species (Fig. A1.3). Time to egg laying was, however, unrelated to the foundresses' host species-of-origin, when treated as a 3-level factor ($G_2=4.1$, $P=0.129$, %Dev=8.09).

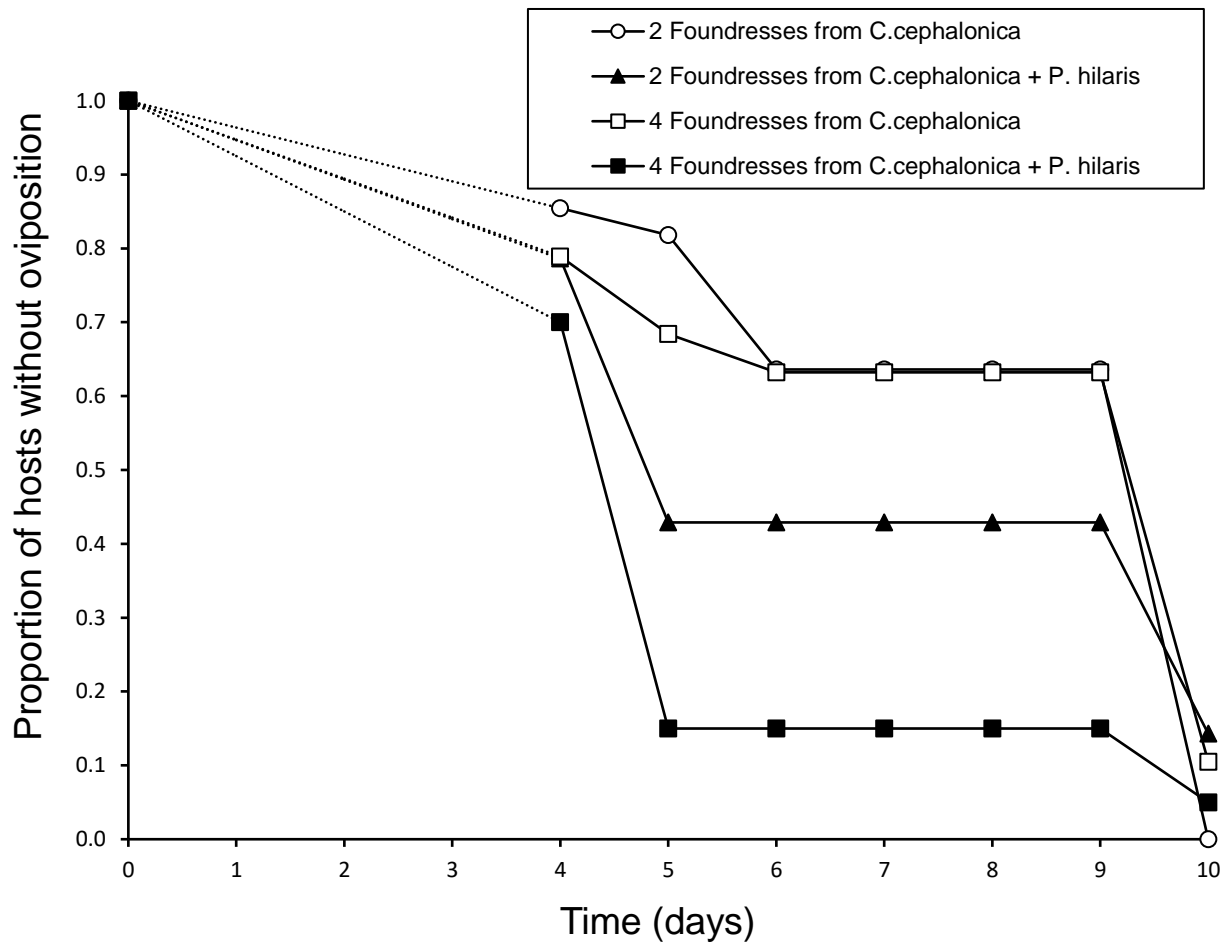


Figure A1.3. The effects of host species-of-origin and foundress number on time taken to oviposition. *Corcyra cephalonica* hosts were oviposited on more rapidly when foundress groups of four comprised individuals reared from different host species.

A1.5. CLUTCH SIZE

There were no effects of host species-of-origin on clutch size (log-linear analysis: 3-level factor: $F_{2,34}=1.05$, $P=0.362$, %Dev=5.81, $n=37$; 2-level factor: $F_{1,58}=1.26$, $P=0.266$, %Dev=2.11, $n=61$), nor was there an interaction with foundress number ($F_{1,57}=0.98$, $P=0.325$, %Dev=1.64). Per-foundress egg production was unaffected by host-of-origin (3-level factor: $F_{2,34}=1.05$, $P=0.362$, %Dev=5.81; 2-level factor: $F_{1,57}=2.15$, $P=0.148$, %Dev=1.57) and there was no interaction with foundress number ($F_{1,58}=0.99$, $P=0.325$, %Dev=0.72). Per-foundress clutch sizes were significantly smaller when foundress numbers were larger ($F_{1,58}=77.27$, $P<0.001$, %Dev=7.18).

A1.6. OFFSPRING DEVELOPMENTAL MORTALITY

Offspring mortality was greatest when co-foundresses had been reared on a mix of host species (logistic ANOVA: 3-level factor: $G_2=5.96$, $P=0.006$, %Dev=25.96, $n=37$; 2-level factor: $G_1=7.17$, $P=0.010$, %Dev=8.53, $n=61$). There was also a significant interaction between host species-of-origin and foundress number ($G_1=8.63$, $P=0.005$, %Dev=10.26), such that the number of offspring produced by co-foundresses reared from different host species was lower when foundress number was higher, whereas the numbers of offspring produced by co-foundresses reared from the same host species were relatively little affected by foundress number (Fig. A1.4).

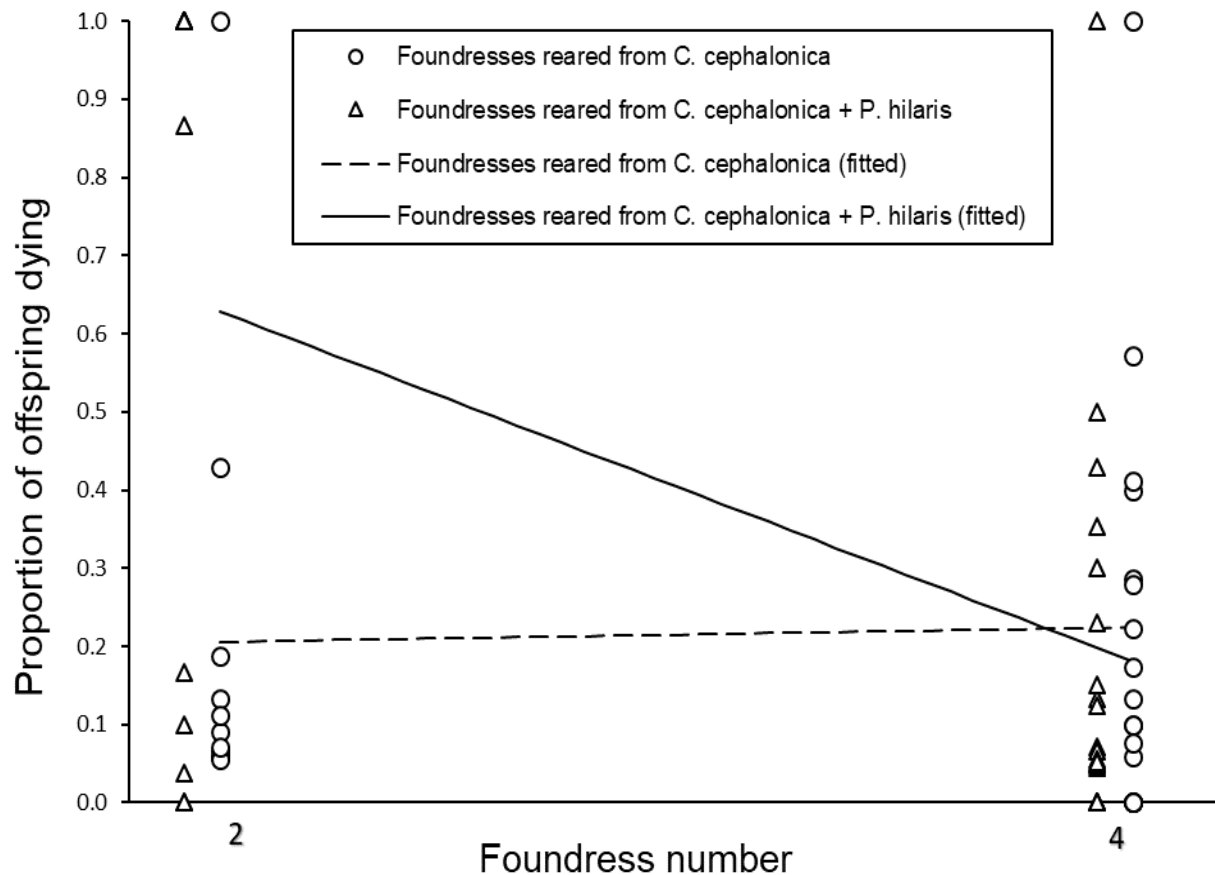


Figure A1.4. The effects of host species-of-origin and foundress number on offspring developmental mortality. Mortality decreased with an increase in foundress number when offspring were produced by co-foundresses reared from different host species, whereas when co-foundresses had been reared from the same host species, mortality was little affected by foundress number.

A1.7. REPRODUCTIVE SUCCESS

The probability of reproductive success (production of ≥ 1 offspring) was unrelated to foundresses' host-of-origin (3-level factor: $G_2=1.11$, $P=0.329$, $\%Dev=2.71$, $n=60$; 2-level factor: $G_1=0.07$, $P=0.789$, $\%Dev=0.07$, $n=80$), but it was significantly affected by foundress number: the probability of success was greater for 4-foundress groups (mean=0.875) than 2-foundress groups (mean=0.375) ($G_1=20.79$, $P<0.001$, $\%Dev=19.84$). Host-of-origin had a marginally non-significant interaction with foundress number ($G_1=3.79$, $P=0.051$, $\%Dev=3.62$) suggesting that reproductive success for co-foundresses reared from the same host species is less sensitive to foundress number variation.

A1.8. BROOD SIZE

Foundresses' host-of-origin had no significant effects on brood size (log-linear analysis: 3-level factor: $F_{2,25}=1.64$, $P=0.217$, %Dev=12.45, $n=26$; 2-level factor: $F_{1,50}=0.01$, $P=0.908$, %Dev=0.024, $n=51$) and there was no interaction with foundress number ($F_{1,48}=0.60$, $P=0.442$, %Dev=1.26). Per-foundress offspring production was not affected by host-of-origin (3-level factor: $F_{2,25}=1.64$, $P=0.217$, %Dev=12.45, $n=26$; 2-level factor: $F_{1,50}=0.14$, $P=0.707$, %Dev=0.17, $n=51$) or by an interaction with foundress number ($F_{1,48}=0.68$, $P=0.414$, %Dev=0.78).

A1.9. BROOD DEVELOPMENTAL PERIOD

Adult offspring emerged within a significantly shorter time when broods were produced by a larger number of foundresses (cohort survival analysis of time to first emergence per brood: $G_1=12.7$, $P<0.001$, %Dev=0.11, $n=65$). Brood developmental period was influenced by host species-of-origin (as a 2-level factor) via a significant interaction with foundress number (host of origin: $G_1=0.1$, $P=0.752$, %Dev=0.18; Interaction: $G_1=18.1$, $P<0.001$, %Dev=17.96, Fig. A1.5): offspring produced by 4-foundress groups that has been reared from a mix of host species emerged earliest. There was no significant effect of host-of-origin fitted as a 3-level factor ($G_2=0.85$, $P=0.654$, %Dev=2.7).

A1.10. ADULT OFFSPRING SIZE

Adult female offspring were smaller when produced by co-foundresses reared from a mix of host species when host-of-origin was treated as a 3-level factor ($F_{2,23}=4.43$, $P=0.025$, $r^2=0.28$, $n=26$). However, in the case of the 2-level factor, there was no effect ($F_{1,47}=1.67$, $P=0.203$, $r^2=0.03$, $n=51$) nor was there an interaction with foundress number ($F_{1,49}=1.22$, $P=0.276$, $r^2=0.02$).

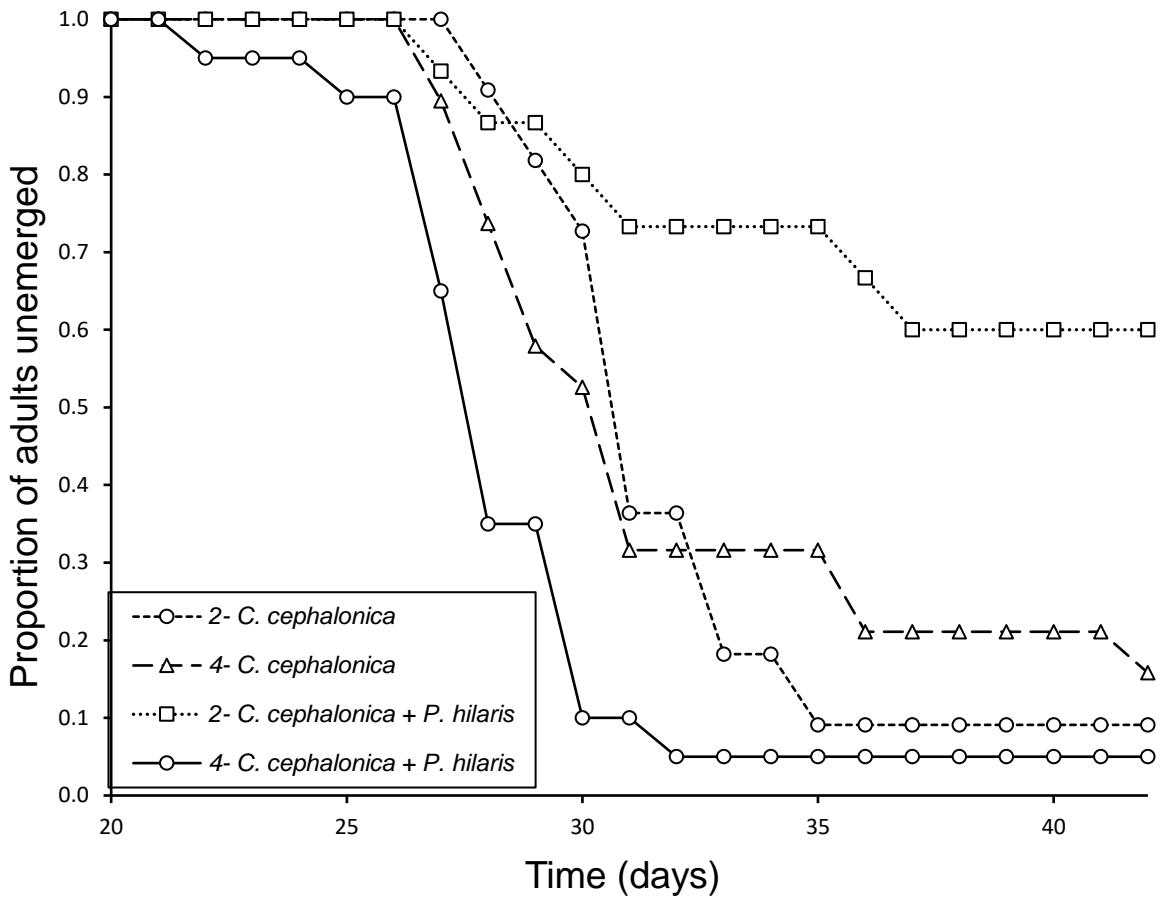


Figure A1.5. The effect of foundress number and host species of origin on post-oviposition brood developmental time. Groups containing four foundresses and reared from two different host species-of-origin produced their offspring within a significantly shorter period compared to those containing pairs of foundresses.

A1.11. SEX RATIO

Sex ratios were not affected by the host species that foundresses developed on (3-level factor: $G_2=0.06$, $P=0.946$, %Dev=0.48, $n=26$; 2-level factor: $G_1=2.23$, $P=0.142$, %Dev=4.38, $n=51$), or by the interaction between host-of-origin and foundress number ($G_1=1.01$, $P=0.320$, %Dev=1.01).

Table A1.1. Summarized host species-of-origin results*

Reproductive trait	Host-of-origin (2-levels)	Host-of-origin (3-levels)	Foundress number (FN)	Interaction with Foundress number (2-levels)
Mortality (proportion)	NS ¹	NS	NS	NS
Mortality (timing)	NS	NS	NS	NS
Paralysis (proportion)	NS	NS	NS	NS
Paralysis (timing)	Foundresses from different hosts attacked faster	NS	Larger groups attacked earlier	NS
Oviposition (probability)	Foundresses from different hosts laid more eggs	NS	Significance via interaction	With an increase of foundress number, co-foundresses from different hosts oviposited more, whereas those from one host oviposited less.
Oviposition (timing)	Foundresses from different hosts oviposited earlier	NS	Larger groups laid eggs earlier	Eggs were laid earlier when there were four co-foundresses that had been reared on different host species
Clutch size (CS)	NS	NS	NS	NS
Per-foundress CS	NS	NS	Smaller with larger groups	NS
Offspring developmental mortality	Greater when foundresses were from different hosts	NS	Smaller groups died more	Mortality was similar among all 4 foundress broods and 2 foundress broods produced by foundresses reared from the same host species, but mortality was higher when offspring were produced by 2 foundresses reared from different host species
Reproductive success (probability)	NS	NS	Higher with larger groups	Marginally NS: Reproductive success for co-foundresses reared from the same host species is less sensitive to foundress number variation
Brood size (BS)	NS	NS	NS	NS
Per-foundress BS	NS	NS	NS	NS
Brood developmental period	Significance via interaction ²	NS	Adults produced by larger groups emerged earlier	Offspring produced by 4-foundress groups that has been reared from a mix of host species emerged earlier
Adult offspring size (FN at oviposition)	NS	Foundresses from different hosts produced smaller adults	NS	NS
Sex ratio	NS	NS	NS	NS

*Significant effects with no footnote retained the same interpretation after multiple-comparisons correction using a false discovery rate of 0.25.

¹NS = No statistically significant influence detected in the initial analysis.

²Non-significant after multiple-comparisons correction.

A1.12 DISCUSSION

Host-of-origin was explored as a proxy for kinship given that the cuticular hydrocarbon profiles of foundresses are likely to be affected by their developmental host and that these profiles may in turn be used in kinship assessment. While we found effects of developmental host on the subsequent reproductive behaviour of foundresses, we also found evidence for intrinsic effects of developing on a beetle larva or moth larva (summarized in Table A1.1). Prior studies of parasitoid reproduction have also concluded that the species a female is reared on has consequences in terms of host-feeding and parasitism capacity (Dai *et al.*, 2014), host acceptance (Henter *et al.*, 1996), longevity and offspring developmental mortality (Corrigan & Laing, 1994), and sex ratios (Othim *et al.*, 2017). Such intrinsic effects generate confounding explanations for apparent effects of kinship. Interpretation of any effects of host-of-origin should therefore be made with this caveat taken into account.

Overall, our results suggest that *S. brevicornis* co-foundresses performed better when reared on different host species; paralysing hosts earlier, ovipositing earlier, laying more eggs and producing adult offspring earlier. These results are the opposite to what would be expected if females use hydrocarbon cues to assess their relatedness to co-foundresses and attune their reproductive behaviours accordingly but would align to expectation if females that develop on beetle larvae perform better than females that have developed on a factitious lepidopteran host. Offspring developmental mortality, however, was higher when co-foundresses had been reared on different host species, possibly due to infanticide. Future studies will be required to investigate this possibility further.

APPENDIX 2:

EFFECTS OF *SCLERODERMUS* FEMALES WING DIMORPHISM

Most *Sclerodermus* females are wingless (apterous) and most males are winged (alate). However, wing dimorphism is common throughout the genus, thus some females are alate and some males are apterous (Kühne & Becker, 1974; Evans, 1978; Papini, 2014; Tang *et al.*, 2014; Wang *et al.*, 2016; Lupi *et al.*, 2017; Wei *et al.*, 2017). As the possession of wings likely enhances capacity for post-eclosion dispersal (Wang *et al.*, 2015) but see (Kühne & Becker, 1974), we expect that alate females more commonly co-found broods with non-siblings than do apterous females, and thus winged and wingless foundresses may differ in their of cooperative behaviour and sex allocation strategies.

Analyses in this Appendix use replicates from Table 1 in the main paper, rows F and G, to test effects of foundress wing morphology for the 2-foundress non-sibling case (Table A2.1).

A2.1. FOUNDRRESS MORTALITY

Foundress mortality (proportion) was not affected by foundresses wing dimorphism (logistic ANOVA: $G_1=1.24$, $P=0.272$, %Dev=3.17, $n=40$ broods), nor was the longevity of foundresses affected by their wing morphology (cohort survival analysis: $G_1=2.6$, $P=0.107$, %Dev=2.64, $n=80$ foundresses).

A2.2. PARALYSIS

The proportion of hosts paralysed was not affected by wing morphology ($G_1=1.31$, $P=0.252$, %Dev=3.07, $n=40$). Nor did wing morphology affect the time taken to paralyse the host (cohort survival analysis: $G_1=0.9$, $P=0.343$, %Dev=1.43).

A2.3. OVIPOSITION

Wing morphology did not affect the probability of oviposition ($G_1=0.02$, $P=0.887$, %Dev=0.14, $n=31$) or the time to oviposition (cohort survival analysis: $G_1=3.00$, $P=0.084$, %Dev=6.6) but note the marginal non-significance suggesting that wingless foundresses may oviposit earlier than winged foundresses.

A2.4. CLUTCH SIZE

Clutch sizes were unaffected by foundress wing morphology (log-linear ANOVA: $F_{1,27}=0.25$, $P=0.622$, %Dev=0.92, $n=29$) nor did wing morphology affect the mean number of eggs laid per-foundress ($F_{1,27}=0.25$, $P=0.622$, %Dev=0.92, $n=29$).

A2.5. OFFSPRING DEVELOPMENTAL MORTALITY

Offspring developmental mortality was unaffected by foundress wing morphology (logistic ANOVA: $G_1=0.13$, $P=0.717$, %Dev=0.49, $n=29$) or by clutch size ($G_1=3.62$, $P=0.062$, %Dev=5.79).

A2.6. REPRODUCTIVE SUCCESS

Foundresses wing morphology did not affect the probability of reproductive success (logistic ANOVA: $G_1=2.71$, $P=0.100$, %Dev=5.11, $n=40$).

A2.7. BROOD SIZE

Brood sizes were not affected by foundresses wing morphology (log-linear ANOVA: $F_{1,23}=1.26$, $P=0.111$, %Dev=10.68, $n=25$) nor did foundresses wing morphology affect mean per-foundress offspring production ($F_{1,23}=0.00$, $P=0.948$, %Dev=0.02).

A2.8. BROOD DEVELOPMENTAL PERIOD

Offspring matured earlier when produced by winged foundresses than when produced by wingless foundresses (cohort survival analysis of time to first emergence per brood: $G_1=6.5$, $P<0.05$, %Dev=15.07, Fig. A2.1).

A2.9. ADULT OFFSPRING SIZE

Adult female offspring were significantly larger when foundresses were wingless than when they were winged (standard ANOVA: $F_{1,23}=6.50$, $P=0.018$, $r^2=0.22$, $n=25$).

A2.10. SEX RATIO

Sex ratios were not affected by foundresses wing morphology (logistic ANOVA: $G_1=0.16$, $P=0.690$, %Dev=0.71, $n=25$).

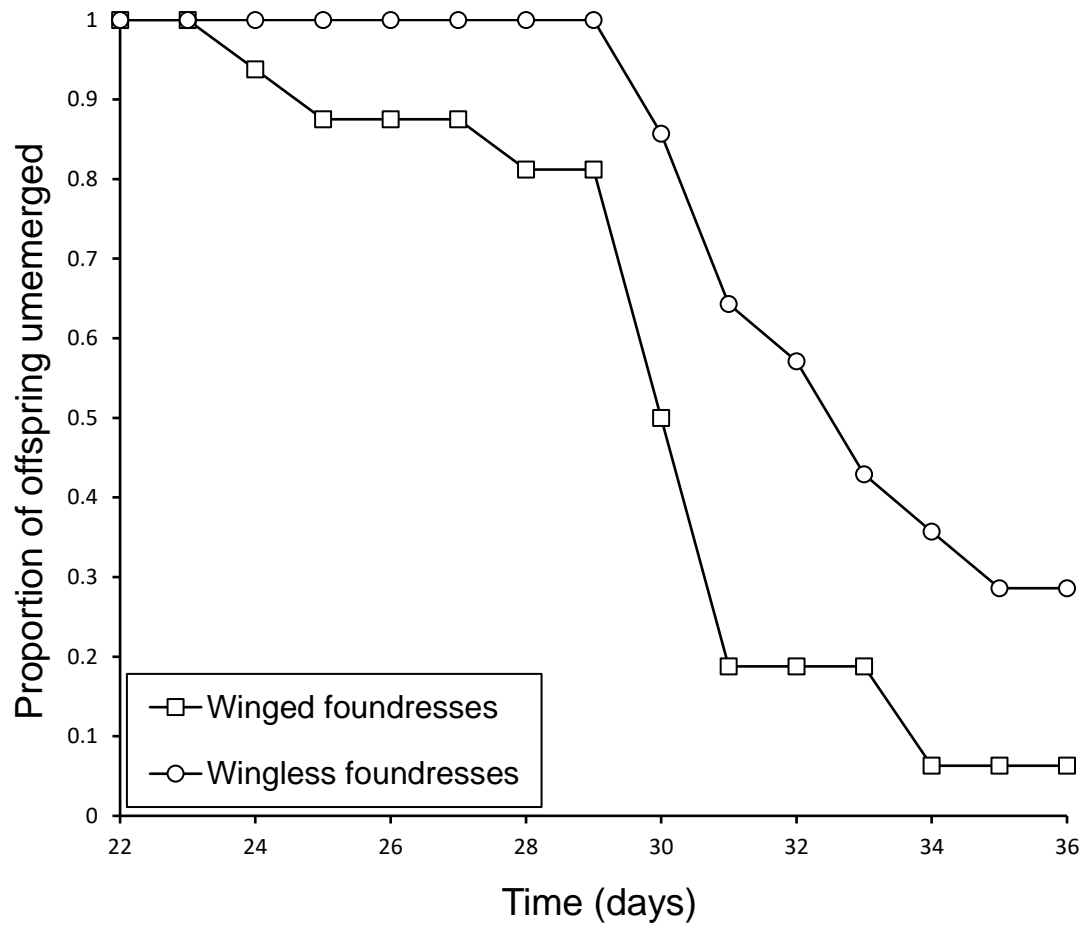


Figure A2.1. The time taken from oviposition to offspring emergence according to foundress wing dimorphism. Offspring produced by winged foundresses taken significantly less time to emerge.

Table A2.1 Summarised wing dimorphism results*

Reproductive trait	Wing dimorphism
Mortality proportion	NS ¹
Mortality timing	NS
Paralysis proportion	NS
Time to paralysis	NS
Oviposition probability	NS
Time to oviposition	Marginal significance ² : Wingless foundresses oviposit earlier than winged foundresses
Clutch size (CS)	NS
Per-foundress CS	NS
Offspring developmental mortality	NS
Reproductive success probability	NS
Brood size (BS)	NS
Per-foundress BS	NS
Brood developmental period	Offspring produced by winged foundresses matured earlier
Adult offspring size (FN at oviposition)	Adults produced by wingless foundresses were larger in size
Sex ratio	NS

*Significant effects with no footnote retained the same interpretation after multiple-comparisons correction using a false discovery rate of 0.25.

¹NS = No statistically significant influence detected in the initial analysis.

²Non-significant after multiple-comparisons correction.

A2.11. DISCUSSION

As alate foundresses are capable of post-eclosion dispersal, they were expected to co-found broods with non-siblings more often than do apterous females, and thus differ in their cooperative behaviour and sex allocation strategies. We found no convincing effects of this dimorphism on the reproductive behaviours assessed. In terms of development, however, the offspring of winged foundresses matured earlier and were smaller than those of wingless foundresses. The scarcity of winged females is typical across *Sclerodermus* species (Tang *et al.*, 2014; Lupi *et al.*, 2017) and precluded us from including all possible combinations of wing-dimorphism and other factors into the experimental design. Current evidence does not suggest that the wing morphology of *S. brevicornis* is strongly associated with host attack behaviour or the degree of cooperation between foundress females.

APPENDIX 3:

PUBLIC GOODS MODELS OF HOST ATTACK

Hamilton's rule states that a trait will spread if its fitness benefits to the recipients (b) multiplied by the relatedness (r) between social partners is greater than the fitness cost (c) to the actor ($rb - c > 0$). There are several approaches to calculating b and c in Hamilton's rule and these are at the core of recent controversy over the general importance of kin selection in evolutionary ecology (Rousset, 2015; Nowak *et al.*, 2017; Van Veelen *et al.*, 2017). While some methods involve regression, the regressions are applied to theoretical models rather than empirical data sets and the slope of the regression represents the benefit, b , and its intercept the cost, c (Gardner *et al.*, 2011; Rousset, 2015; Nowak *et al.*, 2017; Van Veelen *et al.*, 2017).

Here we estimate proxies of b and c from separate empirical (statistical) regressions, one for the effect of host size on offspring production (b) and the other for the effect of host size on the mortality risk to attacking foundresses (c). As benefits and costs are affected by host size, our proxies of b and c are in the form of regression equations rather than being fixed values applied across all host sizes (fitness costs and benefits are thus identified in terms of different currencies (Woodford, 2019). Further, the theoretical regression method (Gardner *et al.*, 2011) involves linearity, because costs and benefits in Hamilton's rule are fundamentally defined in terms of least-squares linear regressions of fitness against genetical traits, while some of the empirical regression results do not because they are more explicitly focused on the causal relationships between traits and components of fitness (e.g. some relationships are polynomial). We refer the reader to the following papers for details on the conceptual issues at stake: Gardner *et al.* (2011), Nowak and Allen (2015), Rousset (2015), Marshall (2016), Birch (2017), Nowak *et al.* (2017), Van Veelen *et al.* (2017), Van Veelen (2018) and Woodford (2019). We emphasise that our purpose is not to critique or to test Hamilton's rule (Bourke, 2014; Van Veelen *et al.*, 2017; Van Veelen, 2018) but to provisionally embrace its qualitative utility to take causal relationships between traits and fitness components and generate testable predictions for the empirical scenario under consideration.

We proceed heuristically in making use of currently available empirical evidence from three prior studies in combination with the public goods framework provided by Hamilton's rule to explore possible influences on *Sclerodermus* host attack decisions. We note that these studies assessed different ranges of host sizes and used different host and wasp species

and that they were not specifically designed to provide data for our current purpose. Nonetheless, all three provide estimates of (or provide information that allows to estimate) the host size dependent probabilities of a foundress dying prior to host suppression ($\approx c$, the cost of attacking a host) and the fitness benefits obtained from paralysed hosts that the actor has successfully attacked ($\approx b$) across a range of host sizes. Where possible we use relationships (regression equations) reported in the original papers; in the remaining cases we estimate them from raw or reconstructed data using generalized linear modelling. We use these relationships to explore, qualitatively, how variation in co-foundress relatedness (r) might affect selection for host attack as a public good (Fig. A3.1; see also Fig. 8 in the main paper). For this, we use an open formulation of Hamilton's rule in which the mechanism generating co-foundress relatedness is undefined (Cooper *et al.*, 2018).

For *Sclerodermus pupariae* attacking *Massicus raddi*, mortality of single foundresses was estimated by logistic regression of data reconstructed from Table 1 in Wei *et al.* (2014) using the mean host weight for each host size class. Foundress mortality increased significantly as host size increased ($G_1=28.6$, $P<0.001$) and was described by the following equation: probability of dying = $1/(1+(1/(\exp((0.03201 \times \text{host weight})-7.2))))$ (Fig. A3.1, panel A). The benefit provided by hosts of a given size is described by the polynomial regression of offspring produced by a single foundress on host size in Wei *et al.*'s Fig. 1c: $y = -0.02x^2 + 0.052x - 3.012$. This relationship peaks at 28.488 and is shown in panel D of Fig. A3.1 divided by this maximum to scale benefits as a proportion. Panel G in Fig. A3.1 suggests that selection favours public goods attack when $r > 0$, except when hosts are large and that, as r increases, the maximum size of a host that a female should attack as a public good also increases.

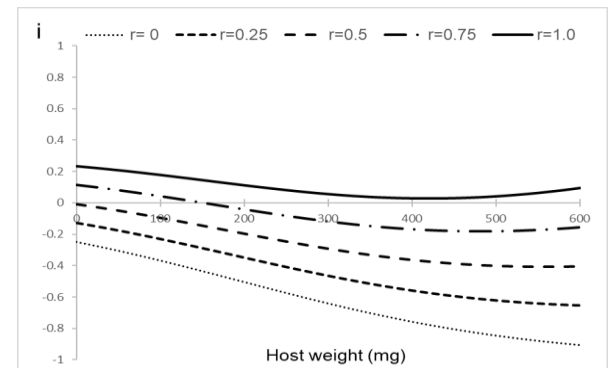
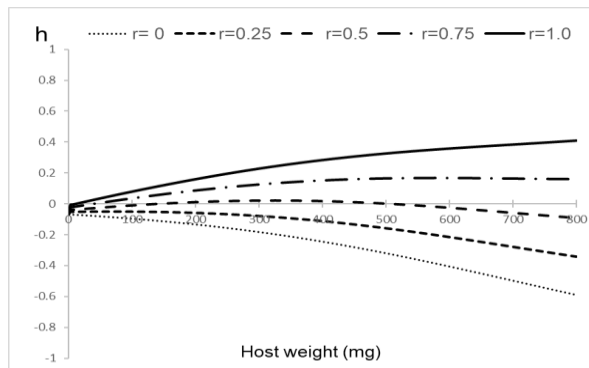
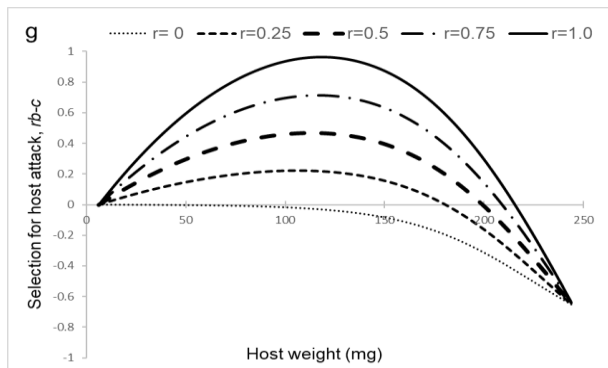
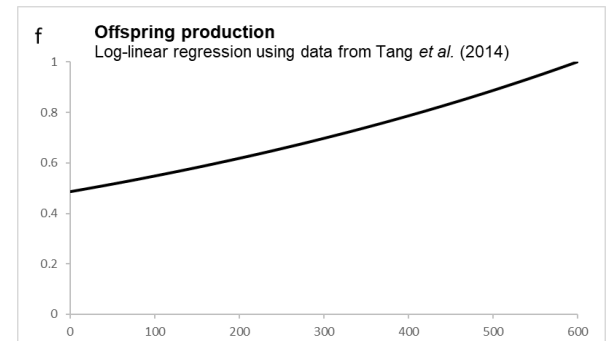
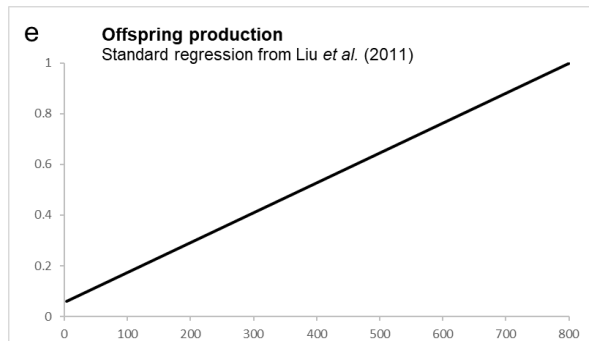
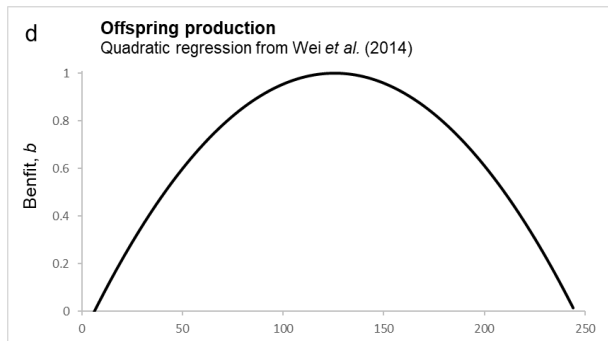
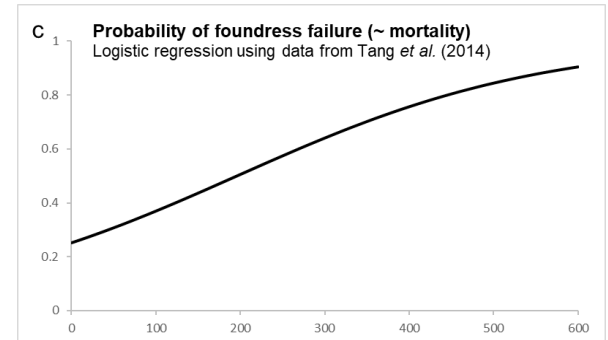
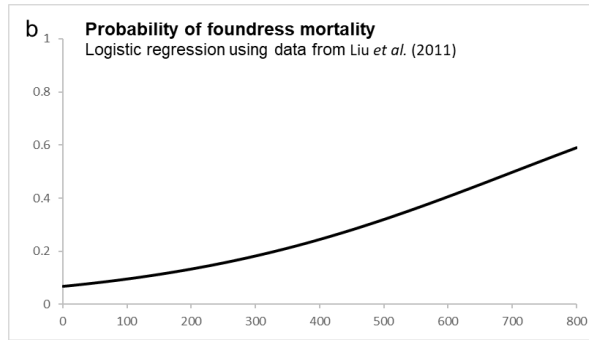
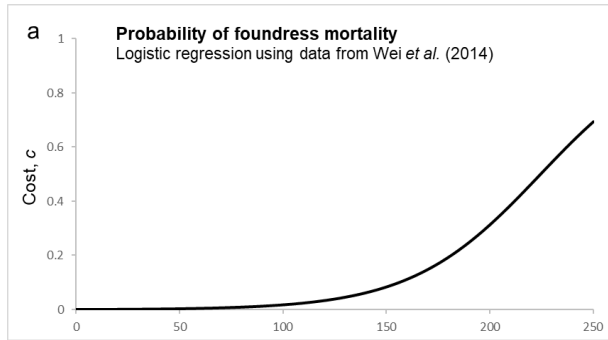
For *Sclerodermus harmandi* attacking *Monochamus alternatus*, mortality of single foundresses was estimated by logistic regression of data reconstructed from Table 1 in Liu *et al.* (2011) using the mean host weight for each host size class. Foundress mortality increased significantly as host size increased ($G_1=5.96$, $P=0.015$) and was described by the equation: probability of dying = $1/(1+(1/(\exp((0.00373 \times \text{host weight})-2.618))))$ (Fig. A3.1, panel B). The benefit provided by hosts of a given size is described by the standard regression equation given Lui *et al.*'s Figure 1c: $y = 0.1203x + 5.6948$. This relationship reaches 101.93 offspring for hosts at the maximum of the range Liu *et al.* considered and benefits, shown in panel E, are divided by this number to scale as a proportion. Panel H of Fig. A3.1, suggests that selection typically favours public goods attack when $r > 0.5$ and that when $r = 0.5$ a female should not attack hosts in the upper half of the weight range studies

as a public good. These values also suggest that very small hosts should not be attacked by any female as a public good.

A second set of estimates for *S. harmandi* attacking *M. alternatus* were obtained using Tang *et al.* (2014) data. Mortality of single foundresses was estimated using host attack failure as a close proxy (given that failure will likely be due to parasitoid death). Foundress mortality increased significantly as host size increased ($G_1=4.23$, $P=0.04$) and was described by: probability = $1/(1+(1/(\exp((0.00557 \times \text{host weight})-1.095))))$ (Fig. A3.1, panel C). The benefit provided by hosts of a given size was obtained using data on adult offspring production across all numbers of foundresses studied. Offspring production increased marginally non-significantly with host weight (log-linear regression using quasi-Poisson errors to correct for over-dispersion: $F_{1,110}=3.12$, $P=0.080$) and was not polynomial (quadratic term: $F_{1,109}=0.92$, $P=0.34$). The regression equation was $= \exp((0.001205 \times \text{host weight})+3.547)$: this is shown in panel G of Fig. A3.1 divided by the maximum predicted brood size for the host size range considered (71.52 offspring) to scale benefits as a proportion. Panel J of Fig. A3.1 suggests that, unless foundresses are genetically identical, selection favours public goods host attack only when relatedness is high (0.75) and hosts <160mg. Under other conditions, females are not expected to pay the private cost of a public good.

Further illustration of combinations of parameters for which Hamilton's rule is satisfied for each other the parasitoid-host associations considered is provided in Figure 8 (main paper).

Sclerodermus pupariae attacking
Massicus raddei



Sclerodermus harmandi attacking
Monochamus alternatus

Figure A3.1. Public goods model of relatedness-mediated host attack. We obtained estimates of host size dependent probabilities of a foundress dying prior to host suppression (cost of host attack, c) and the fitness benefits obtained from hosts paralysed in successful attack (benefit, b) across a range of host sizes and then used these in Hamilton's rule ($rb - c > 0$) to explore how variation in co-foundress relatedness (r) might affect selection for host attack as a public good. These estimates derive from three separate studies, of two different *Sclerodermus*-host species associations, that assessed different ranges of host sizes. In all three cases, foundress mortality increased as host size increased (panels A, B, C). The benefits (scaled to a maximum of 1) provided by suppression of a host were also host-size dependent; either increasing with host size (panels E & F) or in a curvilinear relationship (panel D). The lower panels combine costs and benefits to indicate whether public goods attack is selectively favoured. Panel G suggests public goods attack when $r > 0$, except when hosts are large and that, as r increases, the maximum size of a host that a female should attack as a public good also increases. Panel H suggests that selection typically favours public goods attack when $r > 0.5$ and that when $r = 0.5$ a female should not attack hosts as a public good in the upper half of the weight range studied. These values also predict that very small hosts should not be attacked by any female as a public good. Panel I suggests that, unless foundresses are genetically identical, selection favours public goods host attack only when relatedness is high (0.75) and hosts are small. Under other conditions, females are not expected to pay the private cost of a public good. Overall, in all three examples considered, public goods attack is expected to depend on a combination of host size and relatedness.

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