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Performance of *Sclerodermus brevicornis,* a parasitoid of invasive longhorn beetles, when reared on rice moth larvae

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Abstract

Biological control efficiency can be improved by developing effective mass-rearing systems to produce large numbers of high-quality parasitoids. This study explores an alternative host for rearing *Sclerodermus brevicornis* (Hymenoptera: Bethylidae), a potential biological control agent for the suppression of exotic and invasive wood-boring long-horned beetle populations in the European agroforestry ecosystems. We trial larvae of the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), as hosts for the parasitoid. We quantify the probability and timing of host attack and parasitism as well as reproductive success, offspring production and the characteristics of adult offspring. As *S. brevicornis* is a quasi-social species (multiple females, communally produce offspring broods), we also explore the effects of varying the number of females to which individual hosts are presented, with the aim of determining the optimal female-to-host ratio. As time to host attack can be a limiting factor in *S. brevicornis* rearing protocols, we trial the use of adult females of another species of the bethylid wasp, *Goniozus legneri* Gordh, to paralyse *C. cephalonica* larvae prior to presentation. We identify the conditions within our experiment that maximise offspring production per host and offspring production per adult female parasitoid. We find that *C. cephalonica* is suitable as a factious host and, as it is considerably more straightforward to laboratory-rear than cerambycid species, it is a good candidate for adoption by future *S. brevicornis* mass-rearing and release programmes.

Keywords: Factitious host, parasitoid mass-rearing, life-history, biological control

Introduction

Members of the genus *Sclerodermus* Latreille, 1809 (Hymenoptera: Bethylidae) are small-sized (1.5 – 6 mm) parasitoid wasps (Evans, 1978). There are approximately 81 species worldwide (Gordh & Moczar, 1990; Lanes & Azevedo, 2008). Species of this genus are idiobiont ectoparasitoids (Li & Sun, 2011; Hu *et al.*, 2012), typically of coleopteran larvae. Morphological characteristics of *Sclerodermus* enable them to find and attack their hosts in enclosed spaces, such as feeding tunnels (Kühne & Becker, 1974; Evans, 1978; Yang *et al.*, 2012a; Baena & Zuzarte, 2013; Jiang *et al.*, 2015). Some species are of considerable economic importance as they are used in biological control programmes (Yang *et al.*, 2014) while others may be medically detrimental (Evans, 1978; Papini, 2014; Yang *et al.*, 2014; Skvarla, 2018).

In China, *Sclerodermus* species have formed an integral part of effective and successful forest pest-management programs (Chen & Cheng, 2000; Yang, 2004; Kaishu, 2006; Tang *et al.*, 2012; Yang *et al.*, 2014; Jiang *et al.*, 2015). Among the pest there is the pine sawyer beetle, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae), a vector of pine wood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer) (Lai *et al.*, 2012; Yang *et al.*, 2012b; Zhang *et al.*, 2012; Yang *et al.*, 2013; Yang *et al.*, 2014); the oak long-horned beetle, *Massicus raddei* Blessig (Coleoptera: Cerambycidae) (Wu *et al.*, 2008; Wang *et al.*, 2010; Tang *et al.*, 2012; Yang *et al.*, 2012a); and the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) (Wu *et al.*, 2008; Yang *et al.*, 2012a). Several Asian species of long-horned beetles have invaded Europe (Jucker and Lupi, 2011) and some of the 17 recorded species of *Sclerodermus* (Andre, 1881; Kieffer, 1914ab), have now been reported as associated with these invasive pests (Hérard *et al.*, 2007; Lupi *et al.*, 2014). One of these European natives, *Sclerodermus brevicornis* (Kieffer), was first reported as associated with the long-horned beetle, *Oxypleurus nodieri* Mulsant (Coleoptera: Cerambycidae) approximately one century ago (Kieffer, 1914a) and much more recently, in 2011, was reported in association with the exotic Asian longhorn beetle, *Psacothea hilaris hilaris* (Pascoe) (Coleoptera: Cerambycidae) (Lupi *et al.*, 2014). It was subsequently shown that *S. brevicornis* can be laboratory reared on *P. hilaris hilaris* and two other invasive longhorn beetles species and that it has potential for deployment in biological control programs (Lupi *et al.*, 2017).

While rearing *S. brevicornis* on longhorn beetles is entirely possible, the process of rearing the hosts is time-consuming and physically demanding (Lupi *et al.*, 2015; Lupi *et al.*, 2017). Moreover, *S. brevicornis* foundresses are best left undisturbed for at least four days to successfully paralyse its host, a process that increases the overall duration of parasitoid rearing. The success of parasitoids used in biological control programs is often determined by finding suitable alternative hosts used for their mass-rearing (Lemos *et al.*, 2003; Ramalho & Dias, 2003; Pratissoli *et al.*, 2004). Such hosts should ideally have low production costs and no adverse effects on the reproductive and behavioural performance of the parasitoids (Pereira, 2006). Therefore, attempts have been made to find a suitable factitious host for *S. brevicornis*. Thus far, it has been found that female *S. brevicornis* do not successfully parasitize pupae of the mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), or late-instar larvae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Galleridae) (D.L. unpublished data).

Here we evaluate the potential use of larvae of the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), as host for *S. brevicornis*. Besides being a pest insect, the rice moth is commonly used as a factitious host for rearing around 75 species of natural enemies (Chaudhuri & Senapati, 2017), including parasitoids and predators (Hardy & Blackburn, 1991; Jalali & Singh, 1992; Sahayaraj, 2002; Ballal *et al.*, 2003; Jalali *et al.*, 2003; Lenin & Rajan, 2016; Subandi *et al.*, 2017). Apart from few cases (e.g. Amiresmaeili *et al*., 2018), indeed it is generally easier to produce biocontrol agents on factitious hosts rather than on their natural ones (Kumar & Murthy, 2000). We explore the performance of *S. brevicornis,* when presented with *C. cephalonica* larvae, by quantifying the probability and timing of host attack and parasitism as well as reproductive success, offspring production and characteristics. As *S. brevicornis* is a quasi-social species (multiple females, termed 'foundresses' attack single hosts and produce offspring broods on them communally), we also explore the effects of varying the number of females to which individual hosts are presented, with the aim of determining the optimal female-to-host ratio (Wei *et al.*, 2017). As time to host attack can be a limiting factor in *S. brevicornis* rearing protocols, we trial the use of adult females of another species of the bethylid wasp, *Goniozus legneri* Gordh, to paralyse *C. cephalonica* larvae prior to their presentation to *Sclerodermus* females. *Goniozus legneri* is a gregarious ecto-parasitoid present in several agroecosystems and easy to rear in the laboratory (Legner & Silveira-Guido, 1983; Legner & Gordh, 1992; Steffan *et al.*, 2001; Zaviezo *et al.*, 2007); it is also known to typically attack and paralyse *C. cephalonica* larvae within minutes of presentation (I.H. pers. obs.).

Materials and Methods

All cultures and experiments were carried out in a climate room at 26.0°C ±0.5°C, photoperiod 16:8 (L:D), and 70 ±5% R.H. in a laboratory at the University of Milan, Italy.

Host and parasitoid cultures

A stock culture of *Corcyra cephalonica* was set up in Plexiglas cages (length: 36cm × width: 26cm × height: 25cm) where the adults could fly and mate. To obtain eggs, females were captured and positioned into a small container to allow oviposition. Eggs were then collected and transferred into Petri dishes (15 cm diameter × 2 cm deep) containing a diet used for rearing Pyralid moths (following Limonta *et al.*, 2009). Petri dishes were kept closed until used for experiments or until pupation to obtain new adults for the stock culture.

Sclerodermus brevicornis was maintained on the larvae of the longhorn beetle *Psacothea hilaris hilaris*. Two different systems for rearing *P. h. hilaris* were set up according to the instar (pre-imaginal or adult) following published protocols (Favaro *et al.*, 2017; Lupi *et al.*, 2015). To obtain new offspring of *S. brevicornis*, six to eight newly emerged females were presented with single late instar larvae of the long horned beetle in plastic containers (5 cm diameter × 4 cm deep) on a bed (ca. 1.5 mm) of cork oak granules, following Lupi *et al.* (2017).

A culture of *G. legneri* (Hymenoptera: Bethylidae) was reared on *C. cephalonica*. Individual female parasitoids were presented with single host larvae in glass vials of 10mL with a base of 1.4 mm. The strain of *G. legneri* was obtained from the University of Nottingham, UK, in 2016, and is referred to as 'U' in Khidr *et al.* (2013).

Experiments

All the experiments were carried out using *Corcyra cephalonica* as a host, selecting larvae ranging from 0.030-0.040g. One of two procedures was adopted before *C. cephalonica* were presented to *S. brevicornis*: either *S. brevicornis* females were presented with unparalysed hosts or were presented toa pre-paralysed larvae by a female of *G. legneri, removed* before being presented to *S. brevicornis.* Then, in each replicate, a host was placed into a plastic vial (10cm long × 1.3cm diameter) and either 1, 2, 3, 4, 5 or 6 adult *S. brevicornis* females (foundresses) were added into each vial. The vials were closed with a gauze and cotton wool. To give adequate overall statistical power (Smith *et al.*, 2011), the trial contained the same number of replicates per female at time zero (n=100) but when, prior the paralysis, some parasitoids died inside the vial, this was declassed to the corresponding female group number. The result was that we set up 92, 224, 111, 54, 50 and 54 for foundress numbers of 1, 2, 3, 4, 5 and 6, respectively, giving a total number of 585 replicates. We ensured that a balanced range of pre-paralysed or unparalysed hosts were represented within the replicates of the first four foundress groups. Five- and six-foundress groups were not presented with pre-paralysed hosts.

Vials were observed daily to record host paralysis (occurrence and timing), egg laying (occurrence and time of first oviposition to begin a clutch), pupation (timing of first within a brood), emergence of adult offspring (timing, number and sex) and whether offspring were alate or apterous (*S. brevicornis* is wing dimorphic in both sexes according to Lupi *et al*., 2017). Observations were made attempting not to disturb the wasps and in consequence some offspring may have been overlooked at earlier developmental stages. If a host was oviposited on, it was considered to have been successfully parasitized. Parasitism rate was defined as the proportion of replicates in which oviposition occurred out of total number of replicates in which hosts were paralysed. The production of at least one adult offspring in a replicate was defined as reproductive success.

Statistical analyses

Data were analysed using Generalized Linear Modelling approaches (Aitkin et al., 1989; Crawley, 1993; Faraway, 2006). All analyses were carried out using the statistical software package *GenStat* (version 17, VSN International, Hemel Hempstead, UK).

The explanatory variables 'foundress number' and the 'use of *G. legneri'* (as a pre-host-paralysing agent) were fitted as factors. For some analyses foundress number was initially treated as a factor with six levels but, because there was only variation in *G. legneri* use among 1-4 foundress replicates, analyses including consideration of pre-paralysis excluded 5- and 6-foundress replicates and thus fitted foundress number as a factor with four levels. In some analyses we additional explored results by fitting foundress number as a continuous variable.

The response variables were the parameters recorded during the daily observations and at the end of the experiment. Logistic analyses (GLM) were used to explore binary data on host paralysis, oviposition and foundresses reproductive success; these analyses assumed binomially distributed errors (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. proportion of alate males or females, offspring sex ratio, etc.) assumed quasi-binomial error distributions (the reported test statistic is the *F*-ratio, (Crawley, 1993; Wilson & Hardy, 2002). All logistic analyses adopted a logit link function. Log-linear analyses were used to analyse count data (e.g. number of offspring produced) assuming quasi-Poisson error distributions and a log-link function (Crawley, 1993) . Data derived from integers (e.g. per-foundress brood size) 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.

Parametric cohort survival analyses with censoring (Aitkin *et al.*, 1989; Crawley, 1993) were used to analyse the influences on times-to-events, such as time to host paralysis, oviposition, larval hatching, spinning cocoons and adult offspring emergence.

RESULTS

Probability host utilization and developmental success

Probability of paralysis

The mean probability of a host (which had not been pre-paralysed by *G. legneri*) of being paralysed by *S. brevicornis* was 0.754 (S.E. = +0.021, -0.022, n=411). The probability was greater when there were multiple foundresses (logistic ANOVA: G_5=3.11, P=0.008, %Dev=3.39, Fig. 1). The model was simplified by aggregating foundress number categories to find the minimal adequate model (Crawley, 1993): this showed that there was not a significant difference between the multi-foundress replicates (G_4=0.51, P=0.727, %Dev=0.57, n=350) and that the probability of paralysis was higher among these than among single foundress replicates (G_1=13.51, P<0.001, %Dev=2.95). Similarly, if foundress number was fitted as a continuous variable, the probability of paralysis increased in a curvilinear relationship with increasing foundress number (logistic regression: G_1=3.15, P=0.076, %Dev=0.69; quadratic term: G_1=7.48, P=0.006, %Dev=1.63, Fig. 1).

Probability of oviposition

The overall probability of a presented host of being oviposited on by *S. brevicornis* was 0.610 (SE ± 0.020, n=585). There was a significant interaction between foundress number and pre-paralysis treatment: for unparalysed hosts, oviposition probability increased with foundress number but for pre-paralysed hosts, it declined (logistic ANCOVA, G_3 = 6.50, P < 0.001, %Dev = 2.98, n=481). For hosts that were paralysed (whether by *G. legneri* or by *S. brevicornis*), the probability of subsequent oviposition by *S. brevicornis* was 0.7454 (S.E. = +0.019, -0.020). Again, there was a significant interaction between foundress number and pre-paralysis treatment (logistic ANCOVA: $G_3 = 6.75$, $P<0.001$, %Dev = 4.21, Fig. 2).

Probabilities of development towards adulthood

Among the 357 replicates in which eggs were laid, the probability of at least one egg of hatching was 0.7956 (S.E. = +0.020, -0.022). This probability was significantly lower among eggs laid by five-foundress groups (logistic analysis: $G_5 = 2.49$, $P = 0.029$, %Dev = 3.45). The model was simplified by aggregating foundress number categories to find the minimal adequate model (Crawley, 1993): this showed that there was a no significant difference among foundress groups of 1, 2, 3, 4 and 6 ($G_4 = 0.83$, $P = 0.506$, %Dev = 1.11) and the probability was significantly lower when eggs were laid by five-foundress groups ($G_1 = 9.16$, $P = 0.002$, %Dev = 2.53). Egg hatching probability was not influenced by the use of *G. legneri* ($G_1 = 2.09$, $P = 0.148$, %Dev = 0.79; pre-paralysis × foundress number interaction: $G_3 = 0.04$, $P = 0.988$, %Dev = 0.05).

In replicates with hatched larvae, the probability of some developing to the pupal stage was 0.8416 (S.E. = +0.021, -0.023, n = 284). Larvae produced by four-foundress groups pupated significantly more successfully than those for two or three-foundress groups, whereas pupation success of offspring of other foundress group sizes was intermediate (logistic analysis: $G_5 = 2.79$, $P = 0.016$, %Dev = 5.62). Pupation probability was unaffected by the use of *G. legneri* ($G_1 = 0.00$, $P = 0.947$, %Dev = 0.002; pre-paralysis × foundress number interaction: $G_3 = 0.02$, $P = 0.997$, %Dev = 0.02).

Among replicates in which some offspring reached the pupal stage (n = 239), the probability of at least one adult offspring emerging was 0.983 (S.E. = +0.007, -0.0107); this was unaffected by foundress number ($G_5 = 1.11$, $P = 0.355$, %Dev = 13.60), the use of *G. legneri* ($G_1 = 0.48$, $P = 0.487$, %Dev = 1.24) or their interaction ($G_3 = 1.54$, $P = 0.202$, %Dev = 11.88).

Overall probability of reproductive success

The overall probability of the successful production of at least one adult offspring from a presented host was 0.4017 (SEM = +0.0204, -0.0201). Success did not differ significantly across all foundress number treatments (logistic ANOVA: $G_5 = 1.52$, $P = 0.180$, %Dev = 0.96, n = 585) but there was a positive relationship when foundress number was fitted as a variate (logistic regression: $G_1 = 4.52$, $P = 0.033$, %Dev = 0.57, Fig. 3). Among foundress numbers treatments where pre-paralysis was also varied, there was a significant interaction (pre-paralysis: G_1 = 0.03, P = 0.862, %Dev = 0.005; Interaction: G_3 = 2.72, P = 0.043, %Dev = 1.23, n = 481) such that reproductive success increased with increase of foundress number when *G. legneri* was not utilised but decreased when hosts had been pre-paralysed: this interaction was due to the use of *G. legneri* affecting the probability of oviposition (Fig. 2). Considering only the hosts that were oviposited on, adult offspring were produced in two thirds of the cases (mean = 0.658, SE = +0.025, -0.026); the probability of post-oviposition success was marginally significantly affected by foundress number (G_5 = 2.23, P = 0.048, %Dev = 2.43) but there was no overall trend as foundress number increased (logistic regression: G_1 = 0.16, P = 0.685, %Dev = 0.04) and the probability was unaffected by the use of *G. legneri* (main effect: G_1 = 0.75, P = 0.385, %Dev = 0.21, n = 280; interaction: G_3 = 0.22, P = 0.884, %Dev = 0.18).

Timing of host use and offspring development

Descriptive statistics for timings under all experimental treatment combinations are given in Table 1.

Time to paralysis

Among the 411 replicates in which unparalysed hosts were presented to *S. brevicornis* females, the time to paralyse hosts ranged between 1 and 13 days (mean=3.95, SE= ±0.12) but not all hosts became paralysed (see above). The time taken to paralysis was affected by foundress number (survival analysis with hosts unparalysed by day 13 treated as censors: G_5= 28, *P*<0.001, %Dev = 4.48). Aggregation of factor levels indicated that timing was not significantly different among multi-foundress treatments (G_4= 3, *P*= 0.558, %Dev = 0.47) but was significantly slower in single-foundress replicates (G_1= 25, *P*<0.001, %Dev = 4.15; Fig. 4).

Time to oviposition

The time from host presentation to eggs first observation ranged from 3 to 18 days (mean=7.37, SE= ±0.14) and varied according to the number of foundresses present (G_5 = 35, *P*<0.001, %Dev = 3.77, n = 582); larger foundress groups typically started ovipositing earlier. There was a significant interaction between foundress number and the use of *G. legneri* (G_3 = 22, *P*<0.001, %Dev = 3.01, n = 478); groups of three foundresses took longer to lay eggs on hosts that were pre-paralysed compared to other treatment combinations.

Once a host was paralysed, the time until oviposition, ranged from 1 to 17 days (mean=4.22, SE= ±0.15), varied according to foundress number (G_5 = 88, P<0.001, %Dev = 11.59, n = 476), with eggs typically laid earlier by larger foundress groups (Fig. 5). Times were shorter when *G. legneri* foundresses were not utilised for pre-host-paralysis (G_1 = 18, P<0.001, %Dev = 2.83, n = 394) and, as above, there was a significant interaction between foundress number and the use of *G. legneri* (G_3 = 21, P<0.001, %Dev = 3.3).

Time to egg hatching

The time from when at least one egg was first observed on a host to the first observation of larvae on the host ranged from 1 to 12 days (mean = 4.43, SE = ±0.12). This timing varied across foundress number treatments (G_5 = 23, P<0.001, %Dev = 4.33, n = 354); eggs produced by groups of 5-foundresses typically took the longest time to hatch. Eggs hatched marginally significantly earlier when hosts had not been pre-paralysed by *G. legneri* (G_1 = 4, P = 0.046, %Dev = 0.81, n = 277) but there was no significant interaction with foundress number (G_3 = 0.00, P = 1.00, %Dev = 0.17).

Time to pupation

Pupae were first observed between 4 to 17 days (mean=5.91, SE=±0.16) after the first observation of larvae on a host. Offspring of smaller foundress groups pupated significantly earlier (G_5 = 14, P = 0.0156, %Dev = 3.60, n=282). Time to pupation was unaffected by the use of *G. legneri* (G_1 = 2, P = 0.1573, %Dev = 0.57; interaction with foundress number: G_3 = 2, P = 0.5724, %Dev = 0.72, n=227).

Time to adult emergence

Adult offspring of *S. brevicornis* emerged from pupae between 6 to 26 days (mean=15.56, SE=±0.223) after the first observation of pupae in the brood. This period differed across foundress number treatments (G_5 = 7, P = 0.221, %Dev = 3.99, n = 237). Pupae produced from hosts that were pre-paralysed by *G. legneri* developed to adult offspring within a significantly shorter time (G_1 = 13, P<0.001, %Dev = 6.15, n = 189). There was no significant interaction effect between foundress number and the use of *G. legneri* (G_3 = 7, P = 0.5724, %Dev = 1.27).

Time for egg to adult development

Overall egg-to-adult development ranged between 17 and 40 days (mean = 25.85, SE = ±0.25). Developmental time was significantly shorter when eggs were produced by four-foundress groups ($G_5 = 12$, $P = 0.0348$, %Dev = 2.22, n=354). This time was significantly not affected by the use of *G. legneri* as a pre-host-paralysing agent ($G_1 = 1$, $P = 0.3173$, %Dev = 0.18; interaction with foundress number: $G_3 = 1$, $P = 0.8013$, %Dev = 0.16, n=277)

Number and characteristics of offspring produced

Number of offspring

A mean of 6.34 (SEM = +0.40, -0.38) offspring were produced per host across all 585 replicates. Offspring production did not differ significantly across all foundress number treatments (log-linear ANOVA: $F_{5,579} = 1.95$, P = 0.084, %Dev = 1.66, n = 585). However, among the treatments where pre-paralysis was also varied, there was a significant interaction between pre-paralysis and foundress number (pre-paralysis: $F_{1,474} = 3.84$, P = 0.051, %Dev = 0.77; Interaction: $F_{3,476} = 3.58$, P = 0.014, %Dev = 2.16, n = 481). Overall, a mean of 7.56 (SEM = +0.90, -0.80, n = 174) offspring were produced when hosts were pre-paralysed and 5.84 (SEM = +0.52, -0.48, n = 307) were produced when hosts were not pre-paralysed. The overall mean offspring production per host was at a maximum of 10.20 (SE ± 1.32) when there were six foundresses and the host was not pre-paralysed (Table 2) and (Fig. 8 (a)).

When replicates with no offspring production were excluded, a mean of 15.79 (SEM = +0.63, -0.61) offspring were produced per host. The number of offspring produced was affected by foundress number (log-linear ANOVA: $F_{5,229} = 3.31$, P = 0.007, %Dev = 6.74, n = 235) in a significantly curvilinear relationship (quadratic log-linear regression: $F_{1,234} = 4.07$, P = 0.045, %Dev = 1.72, Fig. 6). Among this restricted set of replicates, production was significantly higher when hosts were pre-paralysed (pre-paralysis: $F_{1,184} = 10.32$, P = 0.002, %Dev = 5.17, n = 187). There was no significant Interaction between foundress number and the use of *G. legneri* ($F_{3,182} = 0.80$, P = 0.494, %Dev = 1.20).

The number of offspring produced per foundress per host across all 585 replicates (i.e. including those with complete reproductive failure) had a mean of 2.78 (SEM = +0.18, -0.17) and was typically lower when foundress number was greater (log-linear analysis: $F_{5,579} = 8.67$, P<0.001, %Dev = 6.97), or when hosts were not pre-paralysed ($F_{1,477} = 4.97$, P = 0.026, %Dev = 0.99, n=481). There was no significant interaction effect between foundress number and pre-paralysis treatment ($F_{3,476} = 2.49$, P = 0.060, %Dev = 1.49). The overall mean offspring production per foundress per host was at a maximum of 7.194 (SE ± 0.854) when there was single foundress and the host was pre-paralysed (Table 2) and (Fig. 8 (b)).

Similar patterns were observed among the replicates in which there was some reproductive success: per-foundress offspring produced per host (mean = 6.91, SEM = +0.34, -0.33) declined with foundress number ($F_{5,229}$ = 37.29, P<0.001, %Dev = 44.88, n=235), or when hosts were not pre-paralysed ($F_{1,183}$ = 8.99, P = 0.003, %Dev = 3.45, n = 187, Fig. 7). There was no interaction between foundress number and *G. legneri* use as pre-paralysis treatment ($F_{3,182}$ = 0.97, P = 0.407, %Dev = 1.12).

Sexual composition of broods

Three of the 235 that produced adult offspring contained male offspring only: a single foundress brood of 13 males, a 2-foundress brood of 15 males and a 3-foundress brood of just one male. As all-male broods typically suggest maternal virginity, and thus a lack of sex allocation control, these replicates were excluded from further sex ratio analysis (following (Hardy & Cook, 1995; Kapranas *et al.*, 2016a). Two broods with exceptionally high sex ratios were also excluded: a single-foundress replicate of 0.89 (a sex ratio three times higher than any other mixed sex brood, suggesting lack of sex ratio control) and a five foundress replicate of 0.90 (several foundresses may have been virgins). Data remained from 31 single foundress broods, 83 2-foundress broods, 40 3-foundress broods, 29 4-foundress broods, 21 5-foundress broods and 26 6-foundress broods.

Brood sex ratios (the proportion of offspring that were male, m/[m+f]) were very strongly female biased (mean = 0.0838, +SE = 0.0051, -SE = 0.0049). Sex ratios were not significantly influenced by pre-paralysis treatment ($F_{1,175}$ = 0.98, P = 0.323, %Dev = 0.40, n = 182) or its interaction with foundress number ($F_{3,177}$ = 0.56, P = 0.641, %Dev = 0.69). Sex ratios were typically more female biased in broods produced by larger numbers of foundresses (logistic analysis: $F_{5,226}$ = 12.44, P<0.001, %Dev = 21.58, Fig. 9). Sex ratios were also affected by brood size and by its interaction with foundress number (brood size: $F_{1,221}$ = 7.77, P = 0.006, %Dev = 2.42; Interaction: $F_{5,225}$ = 4.92, P<0.001, %Dev = 7.64), such that sex ratios declined with an increase of brood size among broods produced by 1, 2, 3 or 5 females and increased among broods produced by 4 or 6 foundresses.

The number of males produced per brood decreased significantly as foundress number increased (log-linear analysis: $F_{5,223} = 9.89$, $P<0.001$, %Dev = 15.16) and increased with an increase of brood size ($F_{1,219} = 49.78$, $P<0.001$, %Dev = 15.26, Fig. 10) as there was a significant interaction between foundress number and brood size ($F_{5,223} = 3.85$, $P = 0.002$, %Dev = 5.90). The mean per-foundress number of males in a brood produced decreased as foundress number increased ($F_{5,223} = 18.54$, $P<0.001$, %Dev = 18.43) and increased with brood size ($F_{1,219} = 4.88$, $P<0.001$, %Dev = 8.52), with generally greater increases when foundress numbers were greater (Foundress number × brood size interaction: $F_{5,223} = 3.32$, $P = 0.007$, %Dev = 3.30).

The proportion of broods that were all females ('virgin broods', according Kapranas *et al.*, 2016b) generally increased with increasing foundress number (logistic analysis: $G_5 = 2.67$, $P = 0.020$, %Dev = 5.06, Fig. 11a) and also decreased with an increase of brood size ($G_1 = 22.21$, $P<0.001$, %Dev = 8.41, Fig. 11b); there was a significant interaction between foundresses numbers and brood size ($G_5 = 3.21$, $P = 0.007$, %Dev = 6.07). Virginity was not significantly affected by the use of *G. legneri* to pre-paralyse the host ($G_1 = 0.04$, $P = 0.845$, %Dev = 0.02, n = 182), nor by its interaction with foundress number ($G_3 = 2.22$, $P = 0.083$, %Dev = 2.91).

Wing dimorphism

Wing dimorphism occurred in both male and male offspring (Table 3). Of the 230 broods containing at least one adult female, there were no instances of exclusively alate (winged) females. In 216 broods, all females were apterous (wingless) while 14 broods contained a mixture of apterous (80.75%) and alate (19.25%) females. Adult males were produced in 170 broods: 164 contained exclusively alate males and six contained a mix of apterous (25.81%) and alate (74.19%) males. Male aptery only occurred when all or most females in a brood were also apterous.

The proportion of females that were alate differed among foundress number treatments ($F_{5,230} = 2.41$, $P = 0.038$, %Dev = 4.73, n = 232), such that adult female offspring produced by single foundress groups contained significantly more alate members. This proportion declined as total brood size increased ($F_{1,226} = 114.78$, $P<0.001$, %Dev = 5.81) and also as the number of females in a brood increased ($F_{1,231} = 10.86$, $P = 0.001$, %Dev = 4.34). There was no significant interaction between foundress number and brood size ($F_{5,225} = 2.06$, $P = 0.072$, %Dev = 4.05) or female offspring number ($F_{5,225} = 1.87$, $P = 0.101$, %Dev = 3.73).

The proportion of males that were alate was generally high but differed significantly according to foundress number, being lowest when broods were produced by three-foundresses ($F_{5,170} = 7.79$, $P < 0.001$, %Dev = 16.60, n = 172). This proportion also declined with an increase in brood size ($F_{1,161} = 17.47$, $P<0.001$, %Dev = 7.44, Fig. 12) but there was no significant interaction between foundress number and brood size ($F_{5,165} = 1.19$, $P = 0.317$, %Dev = 2.53). Alate males were also less common when there were more males in a brood ($F_{1,166} = 14.71$, $P<0.001$, %Dev = 5.37) and there was a significant interaction between foundress number and the number of males in a given brood ($F_{5,165} = 7.90$, $P<0.001$, %Dev = 14.41).

Discussion

We have shown that larvae of the rice moth, *Corcyra cephalonica,* can be utilized as factitious hosts for *Sclerodermus brevicornis*. This is in contrast to a previous attempt to rear *S. brevicornis* on lepidopteran larvae (*Galleria mellonella,* D.L. unpublished data) and it is noteworthy that *Sclerodermus* species normally develop on coleopterans (Gordh & Moczar, 1990). Around 75% of hosts were attacked and paralysed (similar to estimates obtained from *S. brevicornis* attack on equivalently sized long-horn beetle larvae Abdi *et al.* (submitted), and around 60% of all hosts presented had eggs laid onto them (compared to 73% when provided with beetle larvae, Abdi *et al.* (submitted). Oviposition was disrupted by the pre-paralysis of hosts using *G. legneri* (possibly due to changes in host odour). However, the probabilities of paralysis and oviposition increased with foundress number, as did the probability of a host yielding at least one parasitoid offspring as found by Tang and colleagues (2014). Our finding that around half of the presented hosts yielded offspring is consistent with data from *S. brevicornis* reared on larvae of *Psacothea hilaris hilaris* (Lupi *et al.,* 2017) and similar to estimates from other *Sclerodermus* species (Tang *et al.,* 2014).

Timing of offspring production

Lupi *et al.* (2017) reported that the developmental time of *S. brevicornis* on beetle larvae is approximately one month. Our data indicate that overall development times on *C. cephalonica* are similar. The time required to successfully parasitize host decreased with an increase in foundress numbers, as also found by Tang *et al.* (2014). We also found that *S. brevicornis* foundresses took more time to oviposit on hosts (and for their eggs to enclose) when hosts were pre-paralysed by *G. legneri* females: we suggest that this may be related to altered odours when hosts are paralysed. In terms of the timing off parasitoid production, there appear be no major negative effects associated with using *C. cephalonica* as a factitious host.

Numerical offspring production

Across all hosts provided, the numerical production of adult offspring (~6.5 per host) was not affected by the number of foundresses present but, if hosts yielding no offspring were excluded, production was greatest at intermediate numbers of foundresses (peaking at ~18 per host). Our data suggest that to maximise *S. brevicornis* offspring production per foundress per host, hosts should be pre-paralysed and should be presented to a single foundress. Under these conditions around 7.2 offspring per-foundress per host can be recruited into the next generation. This is represented by the parameter *c* in classical discrete-time host-population models: *c* is typically negatively associated with equilibrium host density (Hassell, 2000; Heimpel, 2000), and thus better biocontrol performance. Previous studies on *S. harmandi* (Tang *et al.*, 2014) and *S. pupariae* (Wei *et al.*, 2017) have found that the probability of producing adult offspring from a given host is enhanced when foundress groups were larger than 1. However, the sizes of hosts considered by those studies were substantial (ranging up to 0.64g and 0.24mg, respectively) while the maximum size of *C. cephalonica* hosts studied here was 0.040g: compared to cerambycid larvae, *C. cephalonica* larvae are small hosts.

Offspring properties

Female biased sex ratios are generally a desirable property among parasitoids that are biological control agents because it is females, not males, that attack the target pest (Ode & Hardy, 2008; Li *et al.*, 2010; Boulton *et al.*, 2015). Most adult offspring produced by *S. brevicornis* were females (92%) which accords with prior reports in this species (Lupi *et al.*, 2017) and congeners (Kühne & Becker, 1974; Li & Sun, 2011; Liu *et al.*, 2011; Hu *et al.*, 2012; Tang *et al.*, 2014; Wei *et al.*, 2014; Gao *et al.*, 2016; Kapranas *et al.*, 2016b). It is thought that sex ratio bias in *Sclerodermus* is due to local resource enhancement (LRE), a mutually beneficial female–female interactions that increase the reproductive value of daughters, rather than local mate competition (LMC) which is the more commonly applied expiation of female-biased sex ratios among parasitoids (Tang *et al.*, 2014). Here, we found a slight increase in bias as foundress number increased, while other studies have found the opposite (Tang *et al.*, 2014; Wang *et al.*, 2016). This strongly female-biased sex ratio in *S. brevicornis*, whether caused by LRE or LMC, is considered an asset for biological control programs

(Ode & Hardy, 2008). In addition, cooperation among *S. brevicornis* co-foundresses as a result of local resource enhancement (LRE) is likely to increase the range of successfully attackable host sizes (Tang *et al.*, 2014; Kapranas *et al.*, 2016b), and in consequence, is likely to improve the biocontrol potential of the parasitoid.

Associate with the highly female-biased sex ratio, some broods contained no adult male offspring at all (virgin broods), which is generally undesirable in rearing programmes because, as a consequence of arrhenotocky, unmated females are constrained to produce only male offspring in the following generation. As found in prior studies on *S. brevicornis* (Lupi *et al.*, 2017) and in congeners (Kapranas *et al.*, 2016b), the prevalence of all-male broods declined strongly with brood size and is likely to be associated with mortality of offspring within developing broods. However, we found that virginity was more prevalent when broods were produced by more foundresses, while Kapranas *et al.* (2016b) found the opposite effect of foundress number for *S. harmandi*.

Wing dimorphism was present among the adult offspring of both sexes although the vast majority (*ca.* 99%) of females produced were apterous whereas alate morphs dominated among the males (*ca.* 97%); as observed throughout the genus (Kühne & Becker, 1974; Evans, 1978; Tang *et al.*, 2014; Wang *et al.*, 2016; Lupi *et al.*, 2017; Wei *et al.*, 2017). Apterous males were most common when brood sizes were larger and we found that the production of alate females was associated with single-foundress broods. This is consistent with *S. pupariae* in which the proportion of winged female progeny decreases as foundress number increases (Wang *et al.,* 2016). Often wingless forms are confined to a given emergence site whereas winged morphs have greater potential to colonize hosts at greater distances, which is likely to influence the efficacy of mass release programmes.

Conclusion

Species of *Sclerodermus* are considered among the most effective bethylids used in biocontrol programmes (Yang, 2004; Yang *et al.*, 2014; Jiang *et al.*, 2015). *Sclerodermus brevicornis* is native to Europe and has potential to counter invasion by Asian wood-boring long-horned beetles, especially if mass-reared for subsequent inundative field release (Lupi *et al.*, 2017). However, *S. brevicornis* rearing systems have depended on using larvae of long-horned beetles that require considerable effort and time to maintain in laboratory cultures release (Lupi *et al.*, 2017). *Corcyra cephalonica* is simple and inexpensive to mass-rear and is a commonly used factitious host for a range of parasitoid species, but has not previously been used for *Sclerodermus*. We conclude that, despite being a relatively small Lepidoptera, rather than a large Coleoptera, *C. cephalonica* is suitable for the maintenance of laboratory cultures of *S. brevicornis*. Further, the procedure of pre-paralysis is not required; although it can enhance production of offspring per host, it will be labour-intensive in mass-culture facilities and taking human labour into account will likely lead to its exclusion from optimal rearing protocols. While not all *C. cephalonica* hosts presented to *S. brevicornis* females ultimately yield the next generation of parasitoid offspring, it should be possible to obtain outputs of up to around 10 parasitoids per host, the vast majority of which will be mated females. Figure 8 summarizes the relative outputs for the combinations of culturing conditions trialled. The development of practicable and efficient mass-rearing systems brings the active deployment of European *Sclerodermus* against invasive long-horned beetles closer to implementation.

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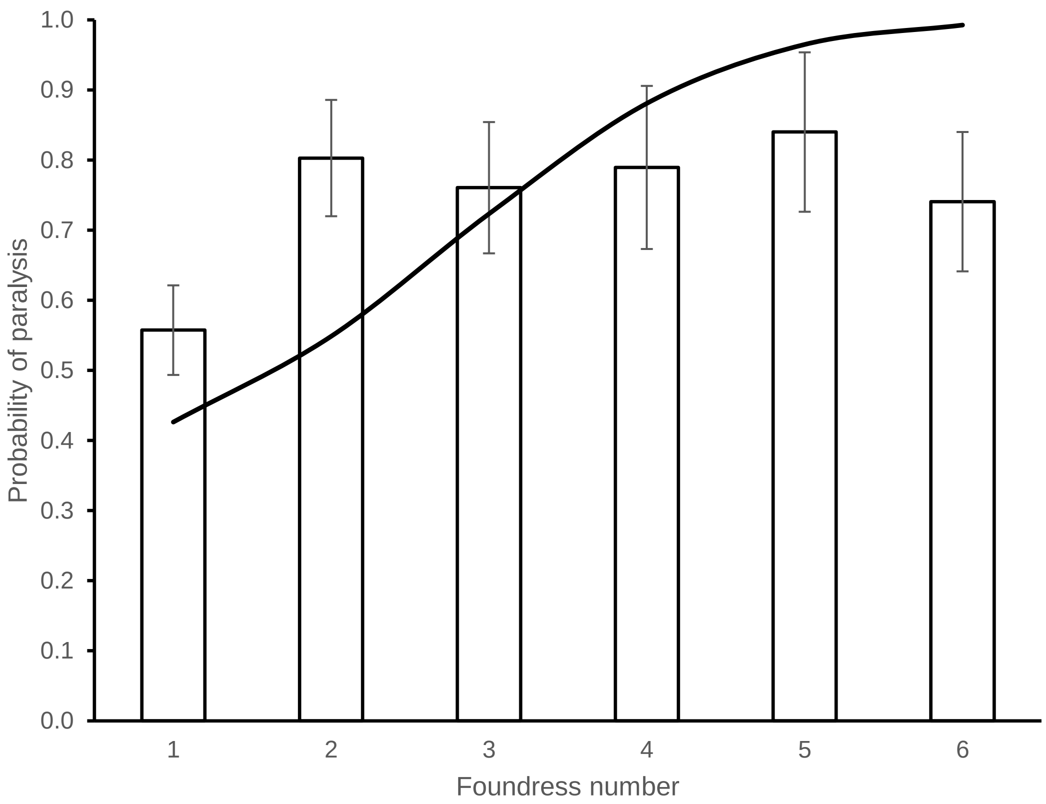

Figure 1. Effect of number of foundresses on the probability of paralysis: The mean probability of a host (which had not been pre-paralysed by *G. legneri*) being paralysed by *S. brevicornis* was greater when there were multiple foundresses**.** The line shows the fitted quadratic logistic regression: probability of paralysis =1/(1+(1/(exp((0.081×foundress number)+(0.137×foundress number^2)-0.515)))).

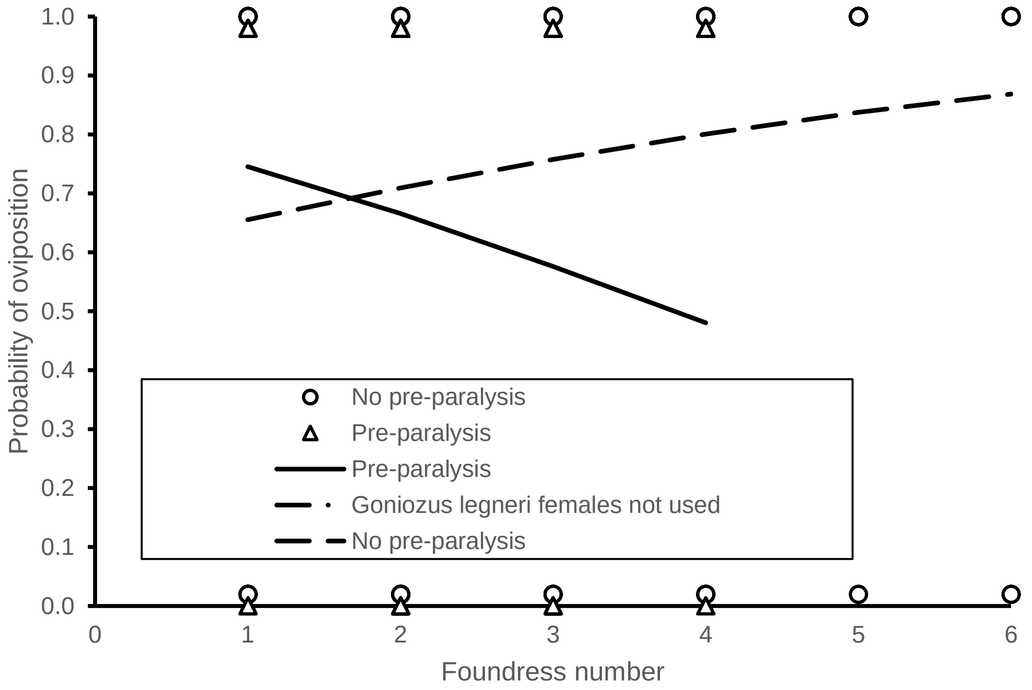

Figure 2. The effect of foundress number on the probability of oviposition: oviposition probability for previously unparalysed hosts increased with an increase in foundress number, but declined when hosts were pre-paralysed by *G. legneri.* Logistic regression lines fitted separately across the range of foundress numbers evaluated.

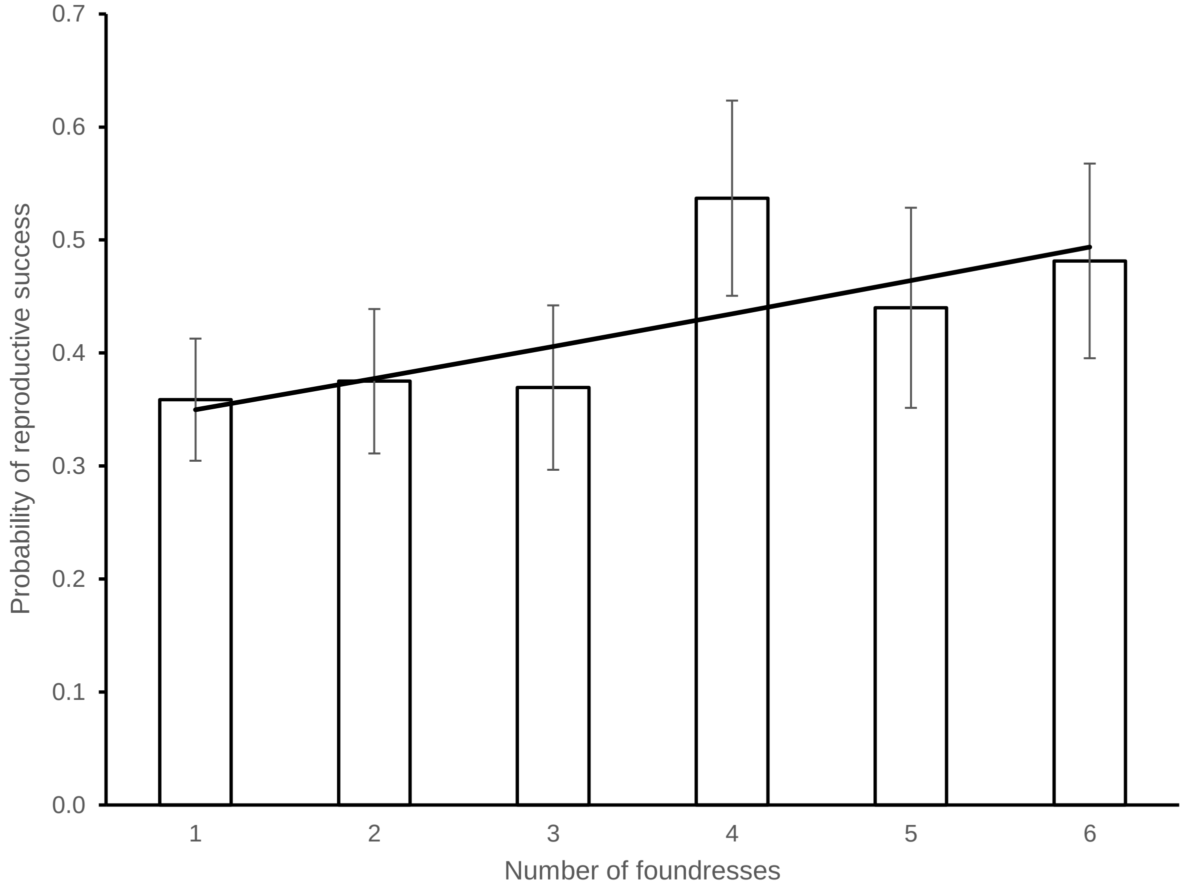

Figure 3. The overall probability of reproductive success. The probability of success did not differ significantly between groups (foundress number treated as a factor) but increased significantly when foundress number was fitted as a variate: logistic regression line, probability =1/(1+(1/(EXP((0.119 × foundress number)-0.739)))).

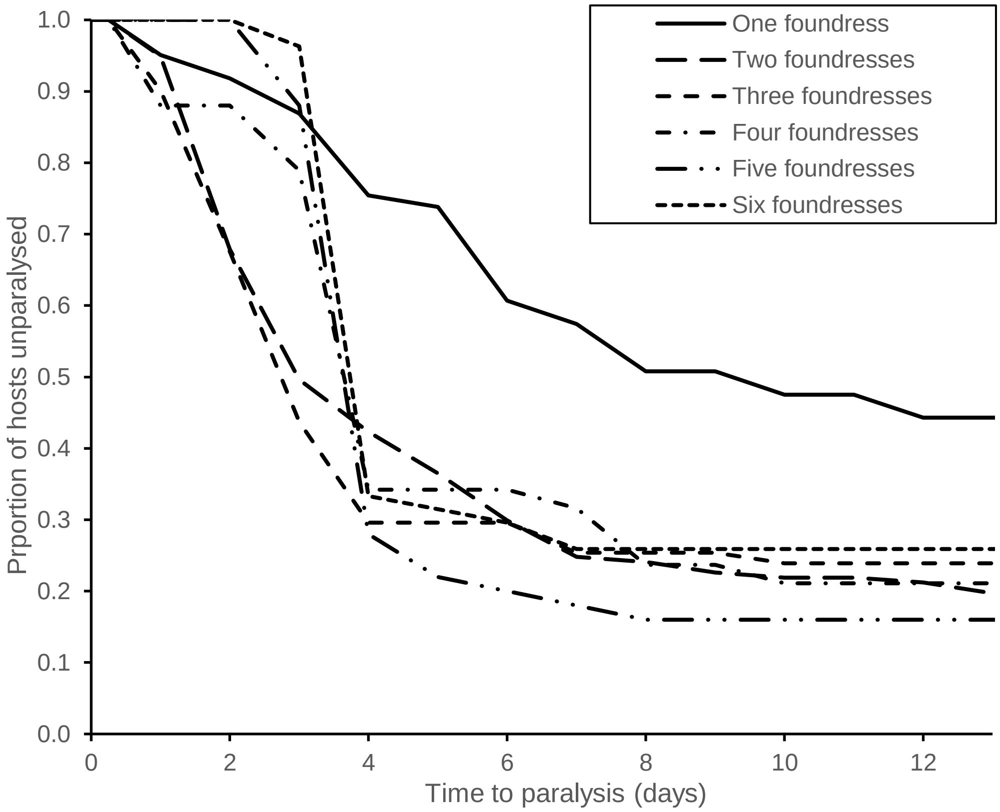

Figure 4. The effect of foundress number on time taken to paralyse hosts. Single foundresses took significantly longer to paralyse hosts than did multiple foundresses. Among multiple foundress replicates, the number of foundresses did not affect timing significantly.

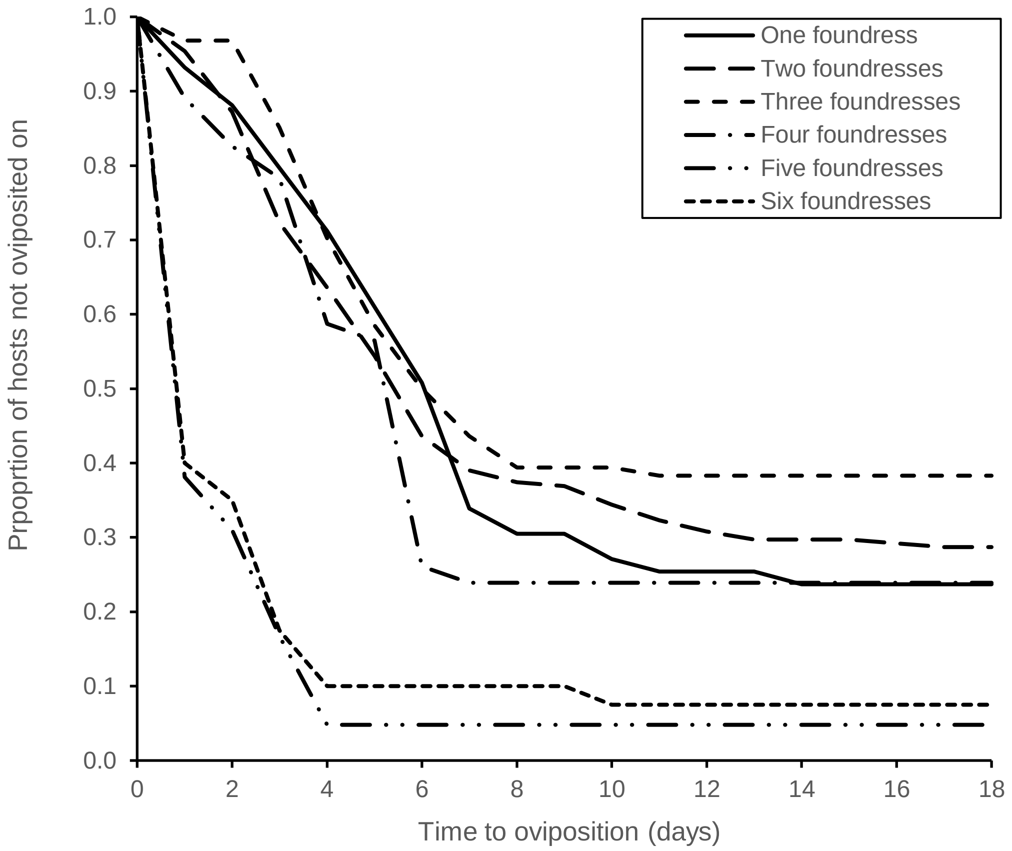

Figure 5. Influence of time to egg laying on paralysed hosts. Smaller foundress groups (1-4) took significantly longer to oviposit on hosts than did groups of 5 or 6 foundresses.

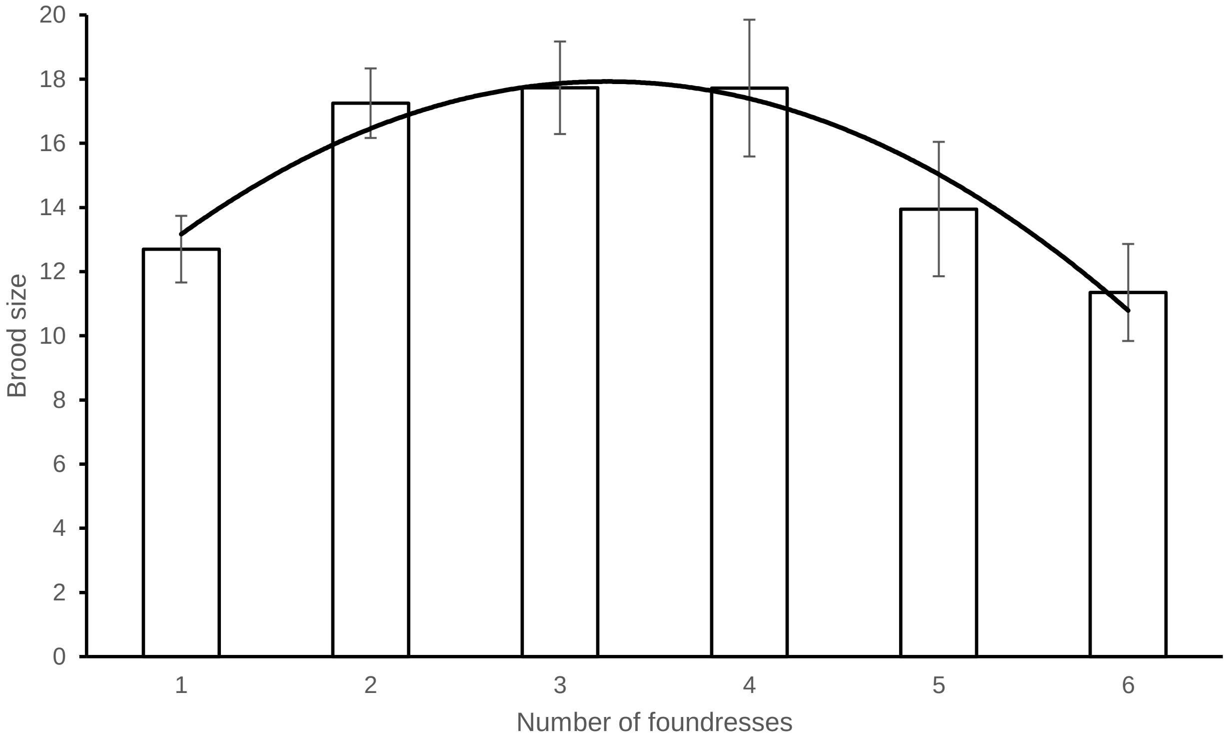

Fig. 6. The number of adult offspring produced. The mean brood size was larger in intermediate sized foundress groups than in smaller or larger groups. Quadratic log-linear regression: brood size = (-0.942 × foundress number^2) + (6.1177 × foundress number) + 7.991).

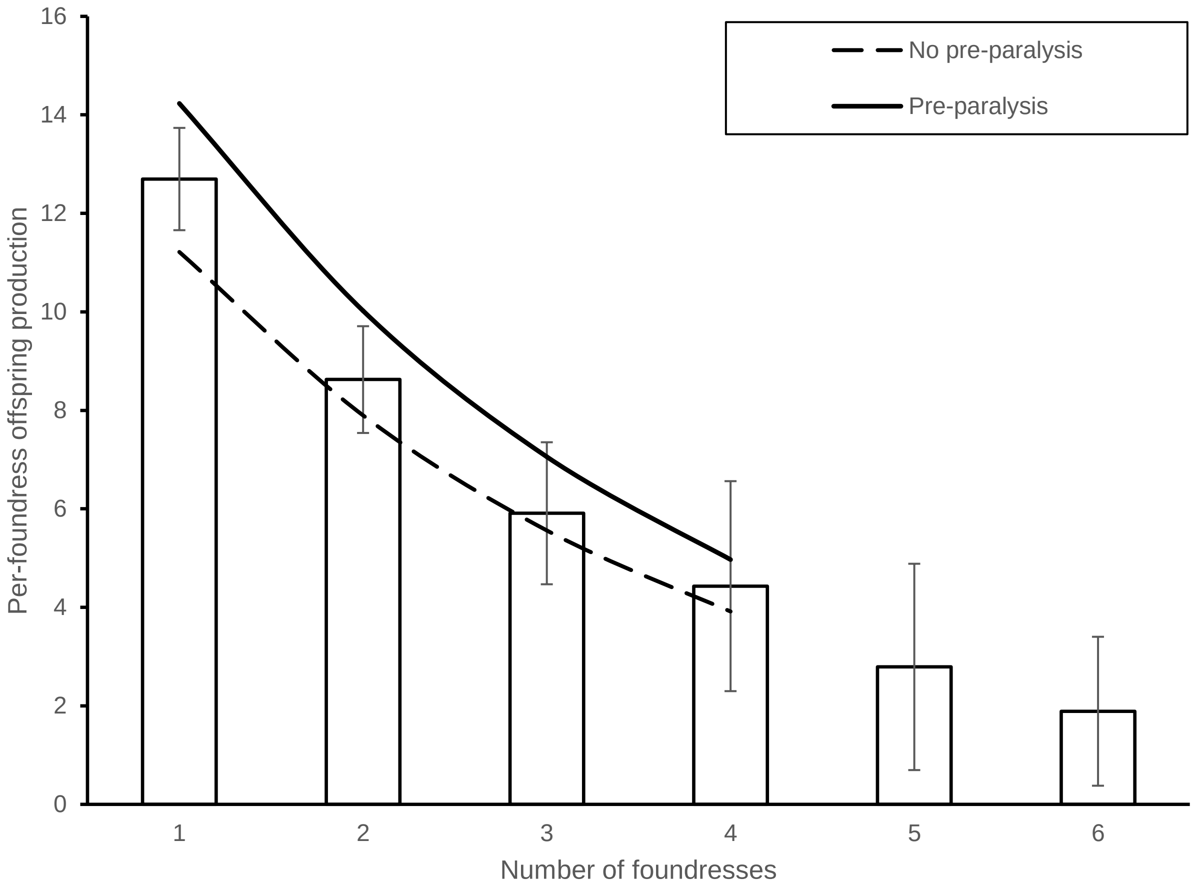

Fig. 7. Effects of number of foundresses and pre-paralysis on per-foundress offspring production. The number of offspring produced per foundress per host (across replicates in which there was some reproductive success) was typically lower when foundress number was greater. Among foundress numbers 1-4, per-foundress production was also lower when hosts were not pre-paralysed by *G. legneri* females.

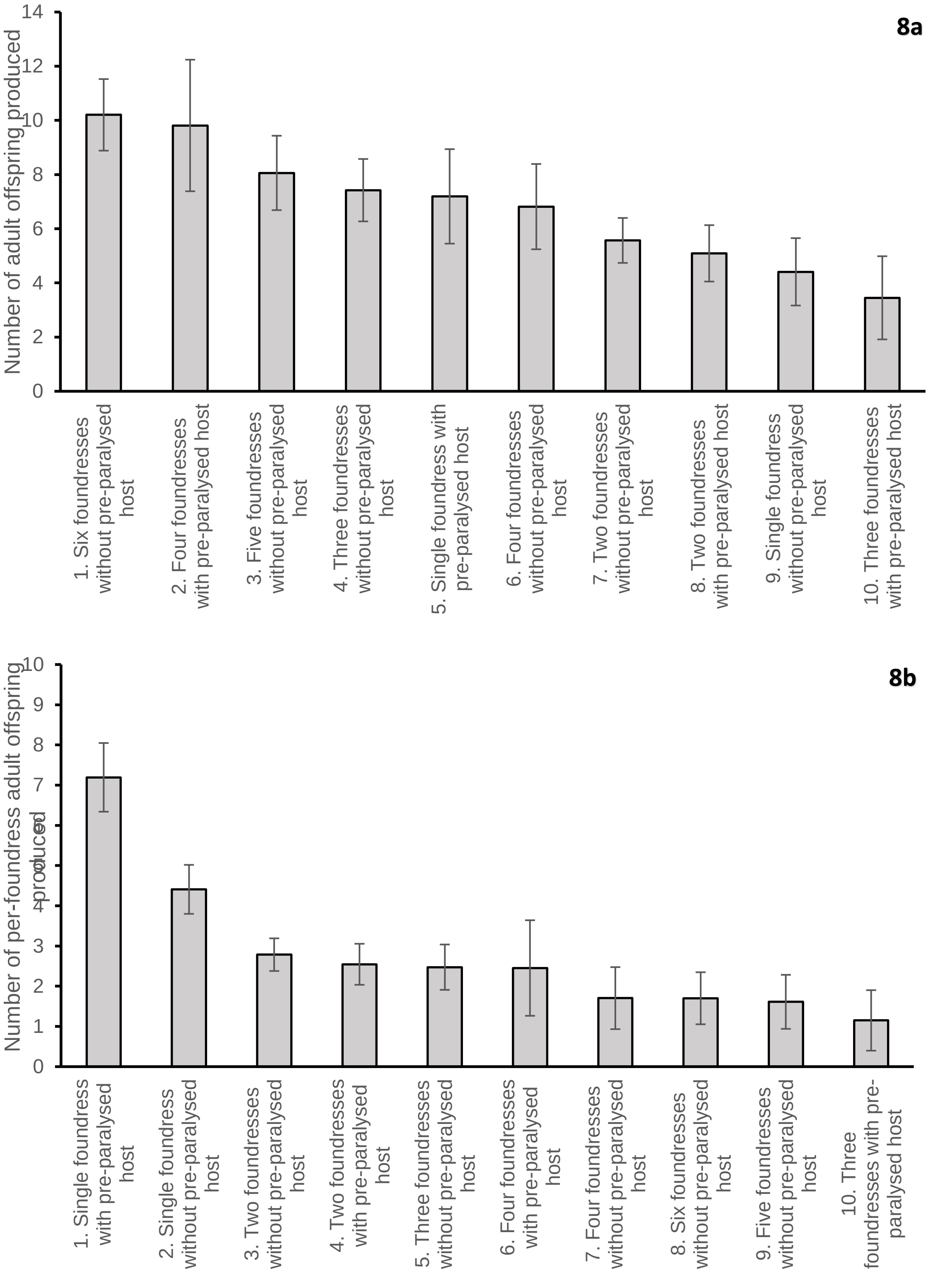

Fig. 8. Offspring production according to the combinations of conditions trialled. Panel (a) shows the total number of adult offspring produced per host whereas **panel (b)** shows adult offspring produced per-foundress per host.

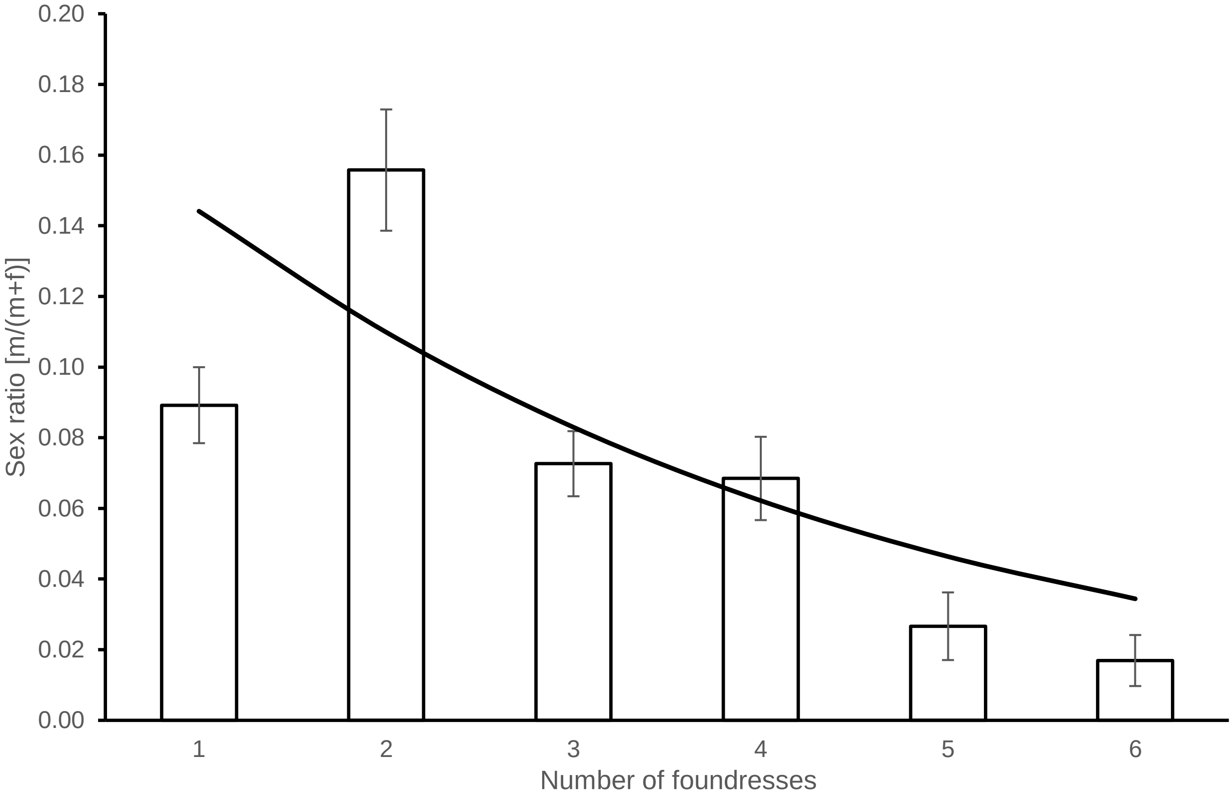

Fig. 9. Sex ratios in relation to foundress number. Broods produced by larger foundress groups were typically more female biased. Logistic regression: sex ratio = 1/(1+(1/EXP((-0.3106 x foundress number)-1.471))).

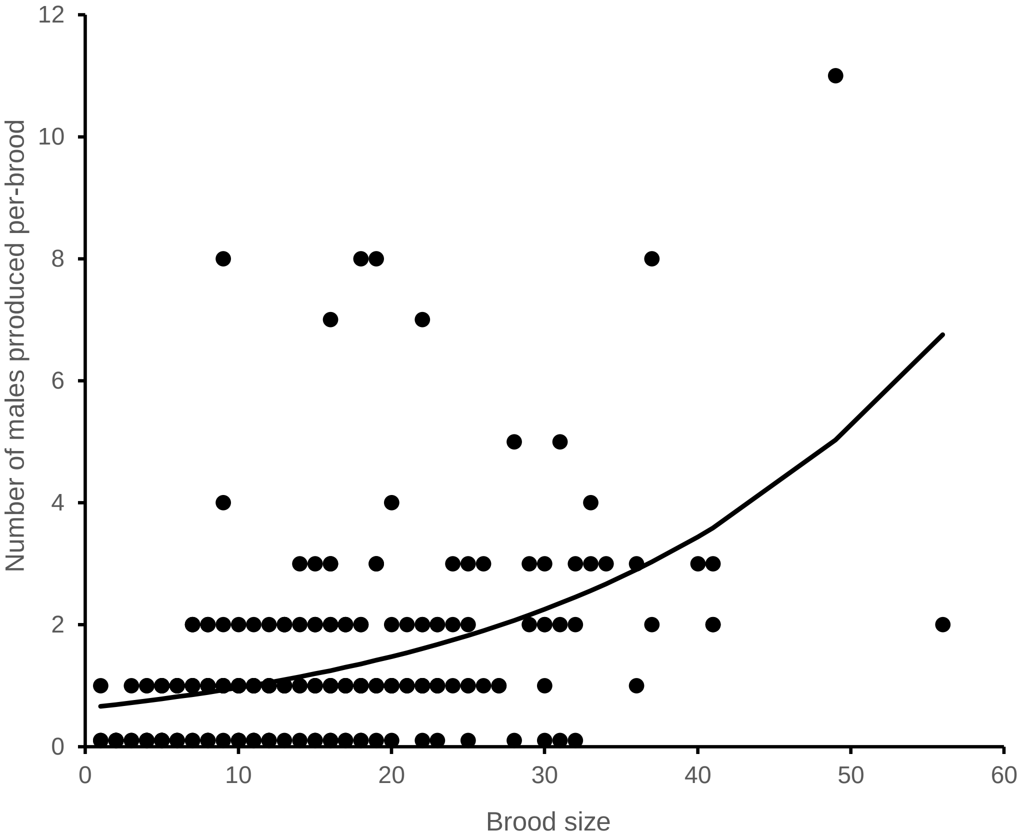

Fig. 10. Effect of brood size on per-brood male production: The number of males produced per brood increased with an increase of brood size

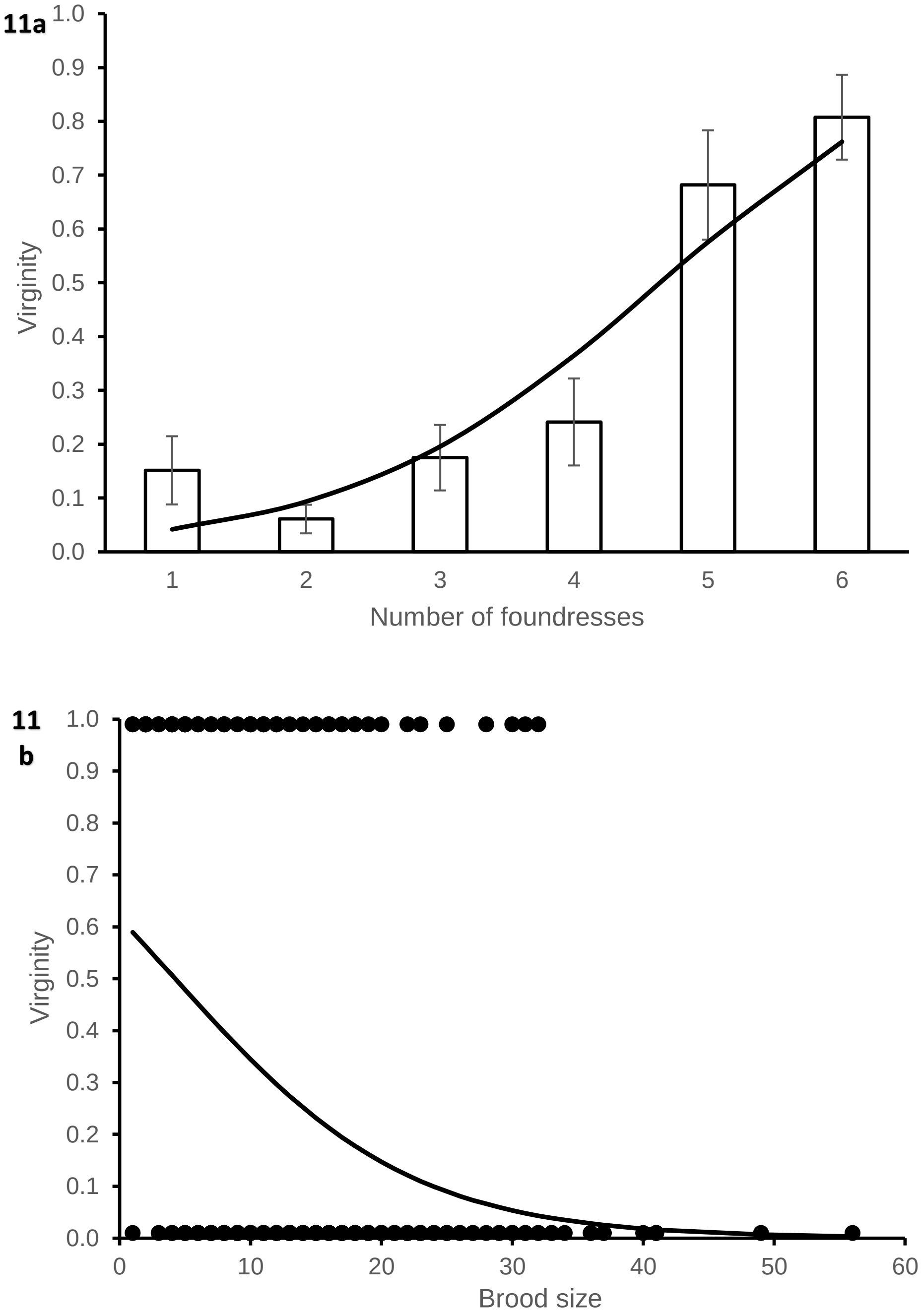

Fig. 11. The prevalence of virgin broods: Panel (a): The proportion of broods that were all females increased with an increase in foundress number. **Panel (b)** virginity decreased with an increase of brood size. Lines were fitted by logistic regression.

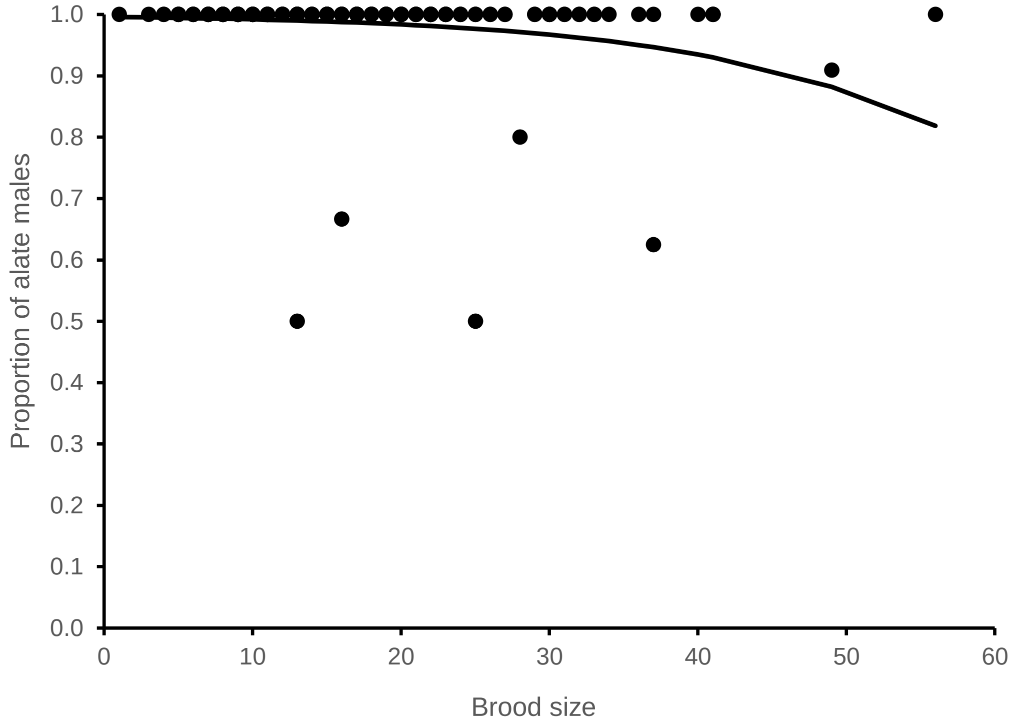

Fig. 12. The relationship between the proportion of winged males and brood size. Line fitted by logistic regression.

Table 1. Times taken for host use and offspring development (Values are mean number of days ± SEM)

Number of Foundresses	***G. legneri* used to pre-paralyse the host?**	**Host presentation-to-paralysis**	**Host presentation-to-oviposition**	**Paralysis-to-oviposition**	**Oviposition-to-hatching**	**Hatching-to-pupation**	**Pupation-to-emergenge**	**Total egg-to-adult development**
1	Yes	1.70 ± 0.21	9.05 ± 0.58	7.25 ± 0.54	6.35 ± 0.80	7.07 ± 0.81	15.08 ± 0.84	26.92 ± 1.05
	No	5.50 ± 0.50	9.50 ± 0.51	4.27 ± 0.47	5.04 ± 0.59	5.10 ± 0.48	17.00 ± 0.58	25.90 ± 0.80
2	Yes	1.08 ± 0.12	8.60 ± 0.33	7.36 ± 0.31	4.54 ± 0.31	6.06 ± 0.42	13.69 ± 0.46	24.28 ± 0.59
	No	3.76 ± 0.24	7.24 ± 0.29	3.42 ± 0.27	4.67 ± 0.28	6.08 ± 0.37	14.00 ± 0.57	24.50 ± 0.75
3	Yes	0.66 ± 0.14	8.60 ± 0.72	6.00 ± 0.67	4.36 ± 0.53	6.00 ± 1.24	16.22 ± 1.01	26.11 ± 0.79
	No	2.98 ± 0.23	7.36 ± 0.39	4.44 ± 0.36	3.63 ± 0.27	5.09 ± 0.44	17.34 ± 0.51	26.16 ± 0.50
4	Yes	2.01 ± 0.27	7.00 ± 0.72	5.00 ± 0.67	4.60 ± 0.54	8.00 ± 0.72	15.70 ± 0.63	28.30 ± 0.30
	No	4.10 ± 0.40	7.36 ± 0.55	3.68 ± 0.52	4.90 ± 0.48	4.84 ± 0.56	17.00 ± 0.71	26.95 ± 0.71
5	No	4.14 ± 0.15	5.78 ± 0.41	1.68 ± 0.38	4.83 ± 0.28	6.09 ± 0.46	17.00 ± 0.81	27.59 ± 0.84
6	No	4.18 ± 0.12	5.89 ± 0.43	1.87 ± 0.40	4.16 ± 0.29	6.31 ± 0.28	16.00 ± 0.54	26.42 ± 0.45
Overall means		3.01 ± 0.24	7.64 ± 0.49	4.50 ± 0.46	4.71 ± 0.44	6.06 ± 0.58	15.90 ± 0.66	26.31 ± 0.68

Table 2. Offspring production according to the combinations of conditions trialled.

Foundress number	**Pre-paralysis treatment**	**Total adult offspring**	**Females**		**Males**		**Per-foundress adult offspring**
			Apterous	**Alate**	**Apterous**	**Alate**	
One	Yes	7.194 ± 1.744	5.839 ± 1.601	0.129 ± 0.084	0.032 ± 0.028	1.194 ± 0.282	**7.194 ± 0.854**
	No	4.409 ± 1.243	3.393 ± 1.141	0.001 ± 0.060	**0.067 ± 0.020**	0.590 ± 0.201	4.409 ± 0.609
Two	Yes	5.092 ± 1.041	4.529 ± 0.956	0.001 ± 0.050	0.001 ± 0.017	0.563 ± 0.168	2.546 ± 0.510
	No	5.569 ± 0.830	5.102 ± 0.762	0.015 ± 0.040	0.001 ± 0.013	0.453 ± 0.134	2.785 ± 0.406
Three	Yes	3.450 ± 1.535	3.025 ± 1.409	0.025 ± 0.074	0.025 ± 0.024	0.375 ± 0.248	1.150 ± 0.752
	No	7.423 ± 1.152	6.634 ± 1.058	**0.169 ± 0.055**	0.014 ± 0.018	0.606 ± 0.186	2.474 ± 0.564
Four	Yes	9.812 ± 2.428	8.000 ± 2.228	0.001 ± 0.116	0.001 ± 0.039	**1.813 ± 0.392**	2.453 ± 1.188
	No	6.816 ± 1.575	6.026 ± 1.446	0.158 ± 0.076	0.026 ± 0.025	0.605 ± 0.255	1.704 ± 0.771
Five	No	8.060 ± 1.373	7.500 ± 1.261	0.020 ± 0.066	0.001 ± 0.022	0.540 ± 0.222	1.612 ± 0.672
Six	No	**10.204 ± 1.321**	**9.593 ± 1.213**	0.001 ± 0.063	0.001 ± 0.021	0.611 ± 0.214	1.701 ± 0.647

Table 3. Wing dimorphism among adult offspring

Sex	**Total offspring**	**Alate**	**Apterous**
Male	306	298 (97.39%)	8 (2.61%)
Female	3346	46 (1.37%)	3300 (98.63%)