

Reproductive performance effects of rearing the quasi-social parasitoid, *Sclerodermus brevicornis* (Hymenoptera: Bethyridae), on a factitious host

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Wasps in the genus *Sclerodermus* are ectoparasitoids that typically attack the larvae of woodboring coleopterans. Interest in these species is increasing as they are used in programs to control longhorn beetle pests of economic importance in China and have invasive pest control potential in Europe. Wasps may be mass reared for field release, but using the target host species can be time consuming and physically demanding. There is thus a need for factitious hosts with lower production costs and that are easier to rear. The present research focuses on *Sclerodermus brevicornis*, which was found in Italy in association with the invasive longhorn beetle, *Psacotha hilaris hilaris*, and can be laboratory reared on this longhorn beetle and on a factitious lepidopteran host, *Corcyra cephalonica*. As it is known that the biology of natural enemies can be influenced by the host they emerge from and that the behavior of *S. brevicornis* is relatively complex due to its degree of sociality (multiple foundress females cooperate to paralyze the host and produce offspring communally), we explored whether, and how, performance and behavioral traits of adult females are influenced by the host species on which they were reared, both when no choice or a choice of current host species was offered. We evaluated the survival of foundresses and their movements between offered hosts and their tendency to form groups with other foundresses according to kinship and host characteristics. We also evaluated the production of offspring and the timing of their development. We found that *S. brevicornis* reared from *C. cephalonica* do have some disadvantages compared with those that have developed on *P. h. hilaris* but also that they recognize, prefer, and can reproduce on *P. h. hilaris*. We conclude that the use of the more convenient factitious host for mass-rearing is unlikely to greatly compromise the potential of *S. brevicornis* to suppress longhorn beetle pests in the field.

Key words: mass rearing, choice test, no-choice test, aggregation behavior

Introduction

Parasitoids in the genus *Sclerodermus* Latreille (Hymenoptera Bethyridae) are of interest to biological pest control practitioners due to their ability to attack longhorn beetle larvae after finding them within infested tree trunks and branches (Chen and Cheng 2000, Yang 2004, Kaishu 2006, Tang et al. 2012, Yang et al. 2014, Jiang et al. 2015). These parasitoids use volatiles to locate suitable habitats and, consequently, their hosts (Yang et al. 2005, Wang et al. 2011, Men et al. 2019). Species in the genus also exhibit quasi-sociality, in which several adult females may cooperate in the attack of large hosts, overcoming its defenses and gaining substantial resources for offspring development (Tang et al. 2014, Wei et al. 2014, 2017, Lupi et al. 2017). Furthermore, in the subsequent communal production and care of large broods of offspring (e.g., Tang et al. 2014, Abdi et al. 2020a, 2020b, Malabusini et al. 2022), foundresses maintain

brood hygiene and assist the larvae during their development and the spinning of cocoons prior to pupation (Wheeler 1928, Hu et al. 2012, Yang et al. 2012). In *S. brevicornis*, the duration of brood development is typically less than 1 month, depending on temperature, host species, and the number of contributing foundresses (Lupi et al. 2017, Abdi et al. 2021). The sex ratios of *Sclerodermus* broods are strongly female biased (Abdi et al. 2020a, 2020b, 2021, Malabusini et al. 2022, Lehtonen et al. 2023). Males are the first offspring to mature (protandry), and they fertilize newly maturing females while they are still within their cocoons (Hu et al. 2012).

In China, *Sclerodermus* spp. have already been evaluated and deployed in the biological control of woodboring beetles in the families Cerambycidae, Scolytidae, Anobiidae, and Bostrichidae. Among these, the most commonly targeted pests are the longhorn beetles, *Monochamus alternatus* Hope, 1843, *Massicus raddei*

(Blessig, 1872), and *Aromia bungii* (Faldermann, 1835) (Wu et al. 2008a, Lai et al. 2012, Tang et al. 2012, Yang et al. 2014, Men et al. 2019), and also the emerald ash borer, *Agrilus planipennis* Fairmaire, 1888 (Buprestidae) (Wu et al. 2008b).

In Europe, current attention is centered on *Sclerodermus brevicornis* Kieffer, which was found in Italy in association with the invasive Asian longhorn beetle, *P. bilaris hilaris* (Pascoe, 1858) (Lupi et al. 2014), and was subsequently successfully reared on this host in the laboratory (Lupi et al. 2017).

However, rearing *S. brevicornis* on *P. b. hilaris* is labor intensive and expensive, and the use of factitious hosts that are easier to rear can assist the mass rearing of the parasitoids. *Sclerodermus* species naturally attack coleopteran larvae and prior work has shown that some coleopterans may be used as factitious hosts. For instance, the mealworm, *Tenebrio molitor* L., can be used for *S. guani* and *S. sichuanensis* (Kai et al. 2006, Zhuo et al. 2016, Guo et al. 2019). *Tenebrio molitor* is, however, not suitable for *S. brevicornis* development (D.L. personal observations). As some other bethylid wasp species naturally attack lepidopterans (Mayhew and Hardy 1998), speculative trials using larvae of the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), were carried out and showed that this species could serve as a factitious host for *S. brevicornis* production (Abdi et al. 2021). A parasitism rate of around 75% was attained using *C. cephalonica*, which is similar to that achieved by *S. brevicornis* when provided with its beetle hosts (Lupi et al. 2017, Abdi et al. 2021). Further aspects related to the capacity of *S. brevicornis* reared on *C. cephalonica* to reproduce and survive low temperature storage are reported by Jucker et al. (2020).

While rearing parasitoids on factitious hosts can be advantageous in terms of short-term savings of space, time, and costs, there may be longer-term negative effects. Changes in parasitoid performance can arise immediately or after several generations of breeding on a given host (van Lenteren 2003, Riddick 2009). For instance, development on different host species may affect the size of developed adults and size may in turn influence subsequent host finding ability, longevity, and fecundity (Hardy et al. 1992, Visser 1994, Harvey 2000, 2005, Luck and Forster 2003, Karsai et al. 2006). Furthermore, parasitoids may use chemical cues associated with the host they developed from to inform their future foraging behaviors (Pomari-Fernandes et al. 2015, Bertin et al. 2017). Finding and recognizing hosts in the field can be a complex challenge for female parasitoids (Fellowes et al. 2023, Quicray et al. 2023) and may be more difficult for parasitoids that utilize cues associated with hosts employed in artificial rearing systems (Gandolfi et al. 2003).

In the current study, we assess whether the behavior and performance of adult female *S. brevicornis*, when presented with hosts of the target species (no-choice tests) or with two different host species (choice tests), are influenced by the species of host on which they have developed (host of origin). As *S. brevicornis* is quasi-social, we study groups of foundress females as well as the behavior of individuals within groups. As recent studies have found that kinship between *Sclerodermus* females influences host attack and reproductive behavior when a single host is presented (Abdi et al. 2020a, 2022b, Guo et al. 2022, 2023), we vary the foundress composition of groups to assess whether the host from which females emerge influences subsequent performance and the distribution of foundresses across hosts when a choice of hosts is available.

Materials and Methods

Host Rearing

The naturally adopted (invasive exotic) host, *Psacotha hilaris hilaris* (Pascoe) (Coleoptera: Cerambycidae) (Asian longhorn beetle),

and the factitious host, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) (rice moth), were used in the present work to assess the biology and behavior of the parasitoid. Both hosts have been shown to be suitable for *S. brevicornis* development under laboratory conditions (Abdi et al. 2020a, 2021, Jucker et al. 2020).

A colony of the xylophagous beetle, *P. b. hilaris*, was reared on an artificial diet since 2013, as described in Lupi et al. (2015), in climate chambers at 26 ± 1 °C, a 16L:8D photoperiod, and a relative humidity of 60 ± 5 %. The *P. b. hilaris* larvae used in the experiments reported here had a mean weight of 0.25 ± 0.0044 g (digital precision balance TE64, Sartorius AG, Germany).

The moth *C. cephalonica* was reared on an artificial diet for more than 30 generations prior to the current study (Limonta et al. 2009, Abdi et al. 2020b). Adults were kept in a plexiglass cage ($36 \times 26 \times 25$ cm), and, in order to obtain eggs, the females were collected and placed in a small glass container where they oviposited. After 2 days, eggs were collected from the bottom of this container using a brush and placed in a Petri dish (15 cm diameter, 2 cm height) filled to a depth of 1 cm with the artificial diet to feed the larvae after hatching. *Corcyra cephalonica* larvae used in the current experiment had a mean weight of 0.029 ± 0.0051 g.

Parasitoid Rearing

The rearing system of *S. brevicornis* was maintained in the laboratory since 2011 following the protocols detailed in Lupi et al. (2015, 2017) and Favaro et al. (2017). Two separate rearing systems were set up: one using the “natural” host, *P. b. hilaris*, and other using the factitious host, *C. cephalonica*, each for more than 30 parasitoid generations. Colonies were maintained in a climate chamber at 25 ± 1 °C, 16L:8D photoperiod, and 60 ± 5 % RH. Adult females were collected shortly after emergence and stored, in groups in vials, in a refrigerator at 4 ± 1 °C for around 15 days (Jucker et al. 2020) until used in the experiment.

Single Host: No-Choice Test

We evaluated host-of-origin effects when mature foundresses were not offered a choice of hosts. Each replicate ($N = 40$) consisted of one *P. b. hilaris* larva placed into a glass vial (8 cm height, 5 cm diameter, closed with cotton wool and a gauze) (Fig. 1). In half of the replicates, 2 *S. brevicornis* females that had developed on the same individual *P. b. hilaris* host were introduced into the vial. In the remaining replicates, 2 females that had developed on the same individual *C. cephalonica* host were introduced. Replicates were maintained inside a climate chamber (26 ± 5 °C, 16L:8D photoperiod, and 60 ± 5 % RH) and were checked once per day, until the death of both foundresses (of no offspring were produced) or offspring emergence, for up to 50 days, under a stereo dissection microscope (MZ 12.5, Leica Microsystems GmbH, Wetzlar, Germany, and Wild Heerbrugg M5A, Leica Geosystems GmbH, Heerbrugg,



Fig. 1. Schematic representation of the starting point of each no-choice test replicate: a vial containing 1 *Psacotha hilaris hilaris* larva and 2 adult female parasitoids.

Switzerland). The following parameters were monitored and recorded: foundress mortality (females were considered to be dead when no movement was detected when stimulated), offspring production (numbers and sexes of emerged adult offspring), and timing (days to host paralysis, days to oviposition, overall days taken for offspring to develop to adulthood).

Two Hosts: Choice Test

We evaluated host-of-origin effects when foundresses were offered a choice of hosts, with the hosts being of different species. Each replicate ($N = 42$) used a 3-sector Petri dish (height: 1.5 cm, diameter: 9.0 cm) in which one *P. b. hiliaris* larva and one *C. cephalonica* larva were placed in separate sectors (Fig. 2). To prevent their movement from the sector, *C. cephalonica* larvae were pre-paralyzed by a female *Goniozus legneri* Gordh (Hymenoptera: Bethyilidae) (maintained in the laboratory since 2016), which was removed once it had stung the host (following Abdi et al. 2021). Four *S. brevicornis* were added into the third sector of each Petri dish, with foundress group composition varied to be either four females that had developed on the same *P. b. hiliaris* host (“4Psaco”, 14 replicates), 4 females that had developed on the same *C. cephalonica* (“4Corcy”, 14 replicates), or 2 females that had developed on a *P. b. hiliaris* plus 2 females that had developed on a *C. cephalonica* (“2P+2C”, 14 replicates) (Fig. 2). Replicates were maintained in a climate chamber at 26 ± 1 °C, 16L:8D photoperiod, and $60 \pm 5\%$ RH.

The “barriers” (low walls) between the sectors within each Petri dish prevented the movement of host larvae between sectors, but parasitoids could pass over them with ease and were consequently free to move within the entire Petri dish and to have contact with either or both of the hosts. To track foundress movement and position, individual females were marked with a dot of nontoxic colored paint (Posca Marking Pen, Japan, tip diameter 0.9 cm) on the middle of the dorsal surface of the pronotum. “Foundress movement” was defined as when a given female was observed in association with a different host than in the previous observation.

Each replicate was observed three times per day (at 10 am, 1 pm, and 4 pm) until offspring pupation, or for up to 50 days, under

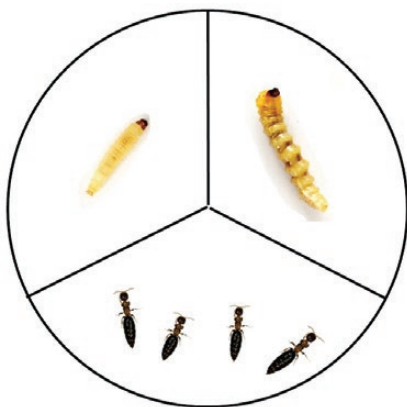


Fig. 2. Schematic representation of the starting point of each choice test replicate using a 3-sector Petri dish, viewed from above. Two sectors contained a host (2 different species) and 4 adult female parasitoids (with varying developmental backgrounds and relatedness) were released into the third. Parasitoids were able to then move freely between sectors while each host remained within its sector.

a stereo dissection microscope. Observations were ceased when all adults emerged or when both hosts in a replicate dried up and no parasitoid offspring survived (from eggs that had been laid on at least one of the hosts); replicates that did not reach a given stage were considered as censors in analyses of timing.

If one host larvae within a replicate died (became desiccated) within the first week of monitoring, it was replaced by a fresh host of the same species and of similar weight. Similarly, when a *S. brevicornis* foundress died during the first week of the trial, it was replaced by a female from the same brood. If at least one host larva died after the first week of monitoring and if *S. brevicornis* females had not laid eggs on either host, the replicate was excluded. If more than one *S. brevicornis* female died between the end of the first week of monitoring and the hatching of larval offspring, the replicate was excluded. Replicates containing 2 pairs of sibling *S. brevicornis* were excluded if one or more of the females died after the first week. These adjustments were made to retain focus on the behavior of “full” groups of females throughout the observation period, rather than to document only the consequences of initial foundress group composition. To obtain the sample sizes given above, excluded replicates were recreated and monitored following the same methodology.

At each observation time, the following information on each *S. brevicornis* female was recorded: parasitoid position within each sector (on the *P. b. hiliaris* larva, on the *C. cephalonica* larva, or “around” [i.e., on neither larvae and thus elsewhere within the Petri dish]); the death of any foundresses; the presence and number of parasitoid eggs, larvae, or pupae on each host; and the numbers and sexes of any mature adults. Brood sex ratio was defined as the number of adult males divided by the total brood size.

Statistical Analysis

We employed generalized linear models (GLMs) and generalized linear mixed models (GLMMs) to explore the effects of experimental treatments on parasitoid performance. GLMs were used for analyses of a single response per replicate (Aitkin et al. 1989), and GLMMs were used when analyses concerned multiple observations per replicate (Bolker et al. 2009). Log-linear analysis, with a log link function, was used for analyses of integer response variables (Aitkin et al. 1989, Crawley 1993) and logistic analyses, with a logit link function, were used for most analyses of proportional response variables (Crawley 1993, Wilson and Hardy 2002). In log-linear analysis and logistic analyses of grouped binary data, quasi-Poisson and quasi-binomial distributions of residuals were adopted, using empirically estimated scale parameters, to take potential over- or underdispersion into account (Crawley 1993, Wilson and Hardy 2002, Hardy and Smith 2023).

For data on the proportion of observations of foundresses on each host, angular transformation was used prior to Gaussian parametric analysis, with an identity link function, followed by post hoc Tukey’s tests with a Type I error rate of <0.05 . Nonparametric analysis using a contingency table was additionally employed to explore the positional association of foundresses with host species according to the host they developed on (we regard this analysis as illustrative rather than formal, see Table 2). Parametric survival analyses were used to identify factors affecting the timing of reproductive events: these were Weibull models with a time-dependent hazard function, with replicates that failed to attain a given developmental stage treated as censors (Aitkin et al. 1989, Crawley 1993, Zhang 2016, Malabusini et al. 2022). All statistical tests were 2 sided. Data were analyzed using the statistical software R (version 4.2.0), except for data in contingency tables that were analyzed using a χ^2 test in Prism GraphPad.

Table 1. Reproduction per host species provided and per replicate according to the hosts of origin of the group of foundresses

Foundress group composition	<i>Psacotheta hilaris hilaris</i> (mean ± SE)	<i>Corcyra cephalonica</i> (mean ± SE)	Totals per replicate (mean ± SE)
(a) Eggs laid			
2P+2C	27.21 ± 5.98	11.00 ± 3.69	38.21 ± 6.06
4Corcy	34.50 ± 6.46	6.50 ± 3.99	41.99 ± 6.54
4Psaco	32.79 ± 5.98	9.07 ± 3.69	41.86 ± 6.06
Overall mean for eggs	31.35 ± 3.48	8.96 ± 2.15	40.33 ± 3.50
(b) Adult offspring produced			
2P+2C	13.27 ± 7.09	3.54 ± 1.66	16.36 ± 6.50
4Corcy	12.75 ± 8.32	4.10 ± 1.74	23.08 ± 7.02
4Psaco	7.00 ± 9.60	6.00 ± 1.94	33.29 ± 6.50
Overall mean for adults	11.6 ± 4.54	4.41 ± 1.00	24.30 ± 3.92

“4Psaco” = 4 females developed on the same *P. b. hilaris*; “4Corcy” = 4 females developed on the same *C. cephalonica*; “2P+2C” = 2 females developed on a *P. b. hilaris* plus 2 developed on a *C. cephalonica*.

Table 2. Summed frequencies of observations of individual foundresses being on hosts of each species

Current host	Host of origin		Totals
	<i>Corcyra cephalonica</i>	<i>Psacotheta hilaris hilaris</i>	
<i>C. cephalonica</i>	834	855	1,689
<i>P. b. hilaris</i>	1,735	1,748	3,483
Totals	2,569	2,603	5,172

Foundresses were observed around twice as often on *P. b. hilaris* host larvae as on *C. cephalonica*, and there was no significant association between the host species that females developed on and current host preference (χ^2 test of 2×2 contingency table: $\chi^2_1 = 0.086$, $P = 0.77$. Note that this analysis is pseudoreplicated and also that pseudoreplication tends to generate false significance: despite this, we find non significance).

Results

No-Choice Test

Foundress mortality

Of the total of 80 foundresses, 28 died during the observation period, and in all cases, dead females had been bitten into 2 parts, although it could not be distinguished with certainty whether it was the *P. b. hilaris* larvae host or another foundress that was responsible. Foundress mortality occurred in 16/40 replicates. The probability of an individual foundress dying was not influenced by the host on which it was reared ($F_{1,82} = 0.86$, $P = 0.35$).

Offspring production

Offspring were produced in 9/40 replicates (5 in replicates with *S. brevicornis* from the *C. cephalonica* rearing system and 4 from the *P. b. hilaris* rearing system). The numbers of adult *S. brevicornis* offspring produced were significantly greater when foundresses derived from *P. b. hilaris* rearing system (*P. b. hilaris*: mean = 46.25, SE ± 6.84; *C. cephalonica*: 16.80 ± 6.12; $F_{1,7} = 10.30$, $P = 0.015$).

Timing of events

After presentation of the hosts, the mean time to host paralysis was 2.35 (SE ± 0.36) days, the mean ovipositional time was 12.70 (+0.46, -0.44) days, and the total time to development (to the first emergence of an adult) was 48.97 (+0.746, -0.735) days. The overall time was calculated considering only those replicates in which *S. brevicornis* reached the egg stage, and replicates that did not reach the adult

stage were treated as censors. The host species on which foundresses had been previously reared did not influence the timing of paralysis, oviposition, or offspring production (cohort survival analyses, with hosts that did not become paralyzed treated as censors; time to paralysis: $\chi^2_1 = 0.78$, $P = 0.38$; oviposition: $\chi^2_1 = 0.73$, $P = 0.39$; development: $\chi^2_1 = 0.23$, $P = 0.63$).

Choice Test

Analyses were performed on data from 40 replicates, as 2 replicates in the treatment “4 Corcy” were excluded due to the death of females between the end of the first week of monitoring and the hatching of larval offspring (see Methods).

Foundress mortality

Some foundresses died during the observation period, and in all cases, dead females had been bitten into 2 parts, although it could not be distinguished with certainty whether it was the *P. b. hilaris* larvae host or another foundress that was responsible (as *C. cephalonica* larvae were pre-paralyzed they were not responsible for foundress death). However, all such deaths occurred before the *P. b. hilaris* hosts were paralyzed, suggesting that deaths were caused by defensive actions of the host. Within the first week of observations, 21 bisected foundresses (13.81% of the total) were counted. In 39.47% (15/40) of replicates, at least 1 foundress was killed during the first week; in 23.68%, 1 foundress was killed, and in 15.79%, 2 foundresses were killed. The probability of an individual foundress dying was influenced by the group composition: mortality was most common (25.00%) when foundresses originated from 2 different rearing systems, and least common (3.85%) when the 4 foundresses were siblings originating from the *P. b. hilaris* rearing system (Logistic GLMM with replicate identity fitted as a random factor: $\chi^2_2 = 7.63$, $P = 0.02$, Fig. 3).

Egg production

Oviposition always occurred on at least one host in each replicate: in 42.5% (17/40) of replicates, eggs were laid on just one host and, among these, oviposition was more common the *P. b. hilaris* host, although not significantly so ($N = 11$, 64.71%; χ^2 test of goodness of fit: $\chi^2_1 = 1.47$, $P = 0.23$). Eggs were laid on both hosts in 57.50% of replicates, and the probability of both hosts being used was not influenced by the type of foundress group (logistic analysis: $\chi^2_2 = 5.45$, $P = 0.07$; note the marginal nonsignificance and that estimates of *P*-values obtained by logistic analysis are approximate rather than

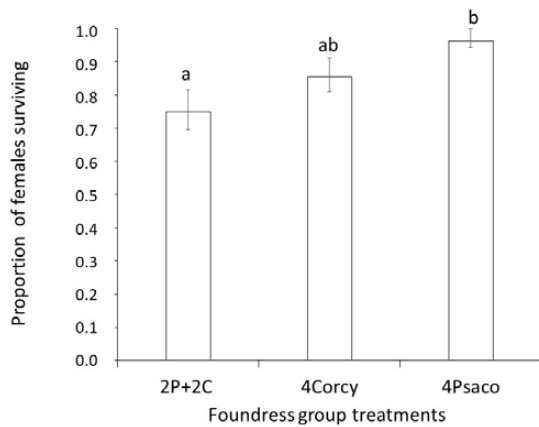


Fig. 3. Survival of *Sclerodermus brevicornis* foundresses during the first week according to foundress group composition (4Psaco = 4 females developed on the same *Psacotha hilaris hilaris*; 4Corcy = 4 females developed on the same *Corcyra cephalonica*; 2P+2C = 2 females developed on a *P. h. hilaris* plus 2 developed on a *C. cephalonica*). The standard errors around the means are asymmetric due to back-transformation from logit-scale estimates. Significant differences are indicated by different letters (Tukey post hoc test).

exact [Crawley 1993]: laying on both hosts was most common when 2 *P. h. hilaris* larvae were provided). Considering all the replicates ($N = 40$), the mean clutch size was 40.33 (SE ± 3.50 , range: 6–86, Table 1a). Clutch sizes were significantly larger on *P. h. hilaris* larvae compared with *C. cephalonica* larvae (GLMM with replicate identity fitted as a random factor: $\chi^2_1 = 436.63$, $P < 0.001$). The total number of eggs laid per replicate was not significantly different between foundress group treatments ($F_{2,37} = 0.10$, $P > 0.05$, Table 1a); nor was the number of eggs laid on each host larva ($\chi^2_2 = 1.27$, $P = 0.53$, Table 1a).

Offspring production

Offspring were produced in 87.50% (35/40) replicates, and those offspring were produced from both hosts in 31.43% (11/35). The mean number of adult offspring per replicate (brood size at adult eclosion, combined across both hosts) was 24.30 (SE ± 3.92 , range: 1–93, Table 1b). Considering only the replicates where adults emerged from only one of the 2 larvae, the number of offspring produced on *P. h. hilaris* hosts was significantly greater than on *C. cephalonica* hosts ($F_{1,19} = 38.32$, $P < 0.001$, Table 1b). There was no significant effect of foundress group composition treatment on the total number of adult offspring produced per replicate ($F_{2,37} = 1.73$, $P = 0.19$), on adults produced from each *P. h. hilaris* larva ($F_{2,22} = 0.15$, $P = 0.86$) or on numbers produced from each *C. cephalonica* larva ($F_{2,26} = 0.49$, $P = 0.62$). Considering only replicates with offspring from only one host larva ($N = 24$), adult offspring production was not significantly affected by the host species utilized ($\chi^2_2 = 0.51$, $P = 0.78$).

Sex ratio

Considering only the 35 replicates in which adult offspring emerged, the mean number of emerged males was 1.43 (\pm SE = 0.22) and ranged from 0 (20% [7/35] of replicates) to 6; no brood consisted entirely of males indicating that at least one foundress in every group had mated. The mean sex ratio per replicate was 0.06 (\pm SE = 0.009) and was not significantly influenced by either foundress group composition ($F_{2,32} = 1.02$, $P = 0.37$) or by the species of host developed on (*P. h. hilaris*, *C. cephalonica*, or both: logistic analysis: $F_{2,32} = 1.74$, $P = 0.18$). Sex ratios declined as brood size increased ($F_{1,33} = 6.44$, $P = 0.02$; Fig. 4).

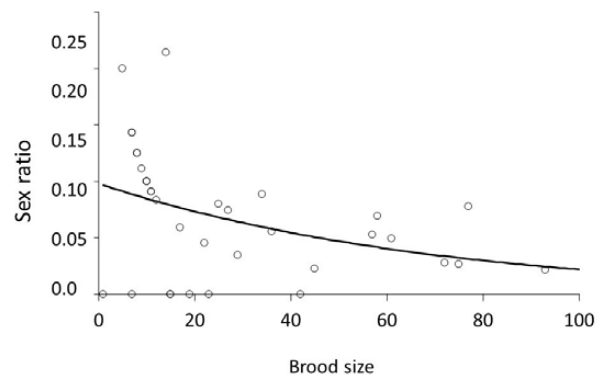


Fig. 4. Sexual composition of broods: relationship between brood sex ratio and the size of the brood, line fitted by logistic regression.

Timing of events

The time taken to oviposit (from the presentation of the hosts to the first egg being laid) on any one host varied between 6 and 30 days, with a mean of 10.78 (\pm SE = 0.57) days. Time to first oviposition was not significantly influenced by foundress group composition (cohort survival analysis, with hosts in which no eggs were laid treated as censors: $\chi^2_2 = 4.28$, $P = 0.12$) or by current host species ($\chi^2_1 = 1.96$, $P = 0.16$). The time to first oviposition was shorter when egg laying occurred on both hosts (12.30 \pm 0.52 days) rather than on just one host (32.79 \pm 0.75 days) in a replicate (cohort survival analysis with censors: $\chi^2_1 = 39.24$, $P < 0.001$).

Analyzing data from only those host larvae on which *S. brevicornis* broods reached the pupal stage (48/80), and considering each host individually, we found that developmental time (from the presentation of the host to adult offspring emergence) varied between 28 and 50 days (mean: 44.43, SE ± 0.72) and was influenced by foundress group composition (cohort survival analysis, with replicates in which no adult emerged treated as censors: $\chi^2_2 = 11.11$, $P = 0.003$; Fig. 5) and by an interaction between foundress group composition and host species (host species main effect: $\chi^2_1 = 0.16$, $P = 0.69$; Host species \times Group composition interaction: $\chi^2_5 = 11.75$, $P = 0.038$, Fig. 6). Parasitoids matured most rapidly (44.70 \pm 1.12 days) in replicates in which the 4 foundresses had developed on *P. h. hilaris* hosts.

Foundress position

We analyzed the positions of individual females at each observation time on each host. As there were multiple records per replicate, replicate identity was included as a random factor (but see Table 2).

We first considered foundresses' positions in terms of the hosts currently presented to them and the host species they had developed on. Across the entire experimental period, females were observed on *P. h. hilaris* larvae about twice as often as on *C. cephalonica* larvae (GLMM with replicate identity fitted as a random factor, $\chi^2_1 = 54.30$, $P < 0.001$, Table 2), and there was no significant effect of foundress group composition ($\chi^2_2 = 1.38$, $P = 0.71$, Table 2). Considering only the period between the presentation of the hosts and the first observation of eggs, females spent significantly more time (percentage of observations) on the *P. h. hilaris* larvae ($\chi^2_1 = 45.09$, $P < 0.001$), and there was no effect of foundress group treatment ($\chi^2_2 = 3.79$, $P = 0.29$). During the periods from oviposition to the first larval emergence (early brood stages) and from the first larval eclosion to the first pupal emergence (late brood stages), there were, again, significant preferences for the *P. h. hilaris* (Early: χ^2_1

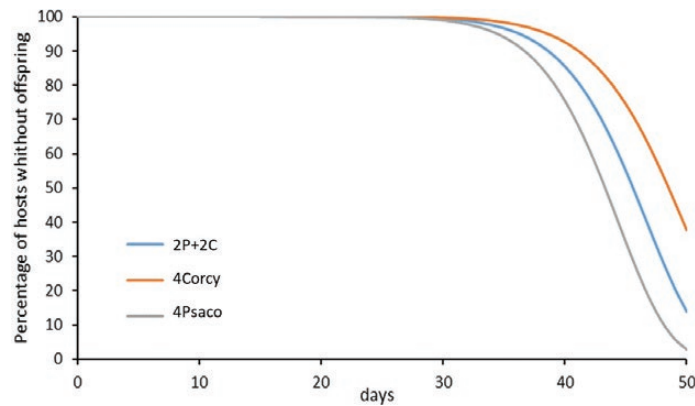


Fig. 5. Cohort survival analysis of *Sclerodermus brevicornis* offspring emergence. Separate lines are shown for each foundress group treatment ("4Psaco" = 4 females developed on the same *Psacotheta hilaris hilaris*; "4Corcy" = 4 females developed on the same *Corcyra cephalonica*; "2P+2C" = 2 females developed on a *P. h. hilaris* plus 2 developed on a *C. cephalonica*); the differences between these were significant (see main text).

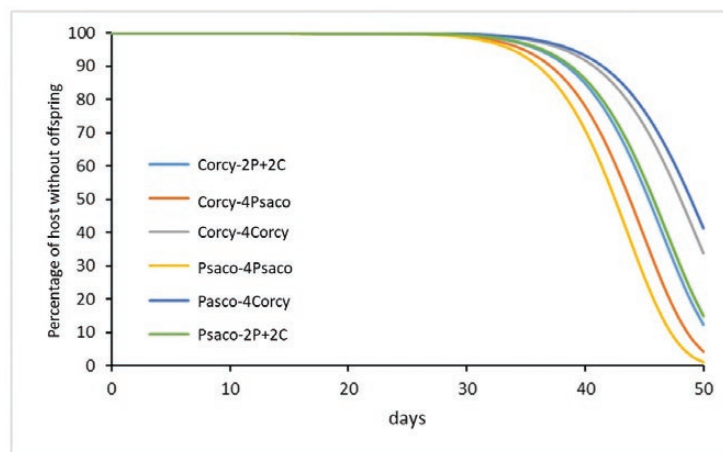


Fig. 6. Cohort survival analysis of *Sclerodermus brevicornis* offspring emergence. Separate lines are shown for each group foundress treatment ("4Psaco" = 4 females developed on the same *Psacotheta hilaris hilaris*; "4Corcy" = 4 females developed on the same *Corcyra cephalonica*; "2P+2C" = 2 females developed on a *P. h. hilaris* plus 2 developed on a *C. cephalonica*) and host species combination ("Psaco" = *P. h. hilaris*; "Corcy" = *C. cephalonica*); the differences between these were significant (see main text).

= 33.43, $P < 0.001$; Late: $\chi^2_2 = 16.83$, $P < 0.001$) and no effect of foundress group treatment (Early: $\chi^2_1 = 6.57$, $P = 0.08$; Late: $\chi^2_2 = 3.20$, $P = 0.36$).

We next considered females' positions in terms of the numbers of females on each host. We calculated the degree of deviation from the null expectation that the 4 females would occupy the hosts in 2 pairs of foundresses (an ideal free distribution, assuming hosts to be of equal quality). Wasps were in this distribution only 3.14% of the time (68/2,163 observations) and deviations were not influenced by foundress group treatment (logistic GLMM, including replicate identity as a random factor: $\chi^2_2 = 0.93$, $P = 0.63$). For *P. b. hilaris* hosts, there were similar percentages of occurrence of single females (28.38%), 2 females (29.63%), and 3 females (28.73%) on one host (Table 3). For *C. cephalonica* hosts, females were most likely to be observed alone on a host (55.17% of observations, Table 3).

Foundress group treatment levels varied both in terms of foundresses developmental backgrounds and in terms of the relatedness of foundresses within groups. Using the data from replicates with 2 pairs of sibling foundresses, we explicitly considered foundresses' positions in terms of their relatedness to other foundresses by calculating a sibling aggregation score from each observation. This had

a value of zero if none of the females were observed together on a host with their sibling, rising to 1 if all females were with their sibling. The overall mean was 0.244 (\pm SE = 0.016), indicating that in around one quarter of the observations, sibling foundresses were associated with each other on the same host. This is lower than the null expectation of 0.47 (assuming all spatial arrangements of the 4 wasps are equally likely), suggesting that siblings tend to dissociate from each other.

Foundress movements

During the entire observational period, from host presentation to pupation of offspring broods, females were observed to move between the presented hosts. The overall mean number of movements per female in a replicate was 1.43 (\pm SE = 0.13), with a maximum of 10 movements by an individual female. The total number of foundress movements within replicates were influenced by foundress group composition (log-linear GLMM with replicate identity fitted as a random factor, $\chi^2_2 = 7.66$, $P = 0.02$), being most frequent when foundresses originated from the *P. b. hilaris* rearing system (Fig. 7). Assessing the movements of each individual foundress similarly showed that foundresses reared on *P. b. hilaris* moved more often (GLMM $\chi^2_1 = 7.94$, $P = 0.005$).

Table 3. Frequencies of foundress numbers observed per host

Number of foundresses on host	<i>Corcyra cephalonica</i>	<i>Psacotha hiliaris hiliaris</i>	Overall
1	550	409	959
2	295	427	722
3	123	414	537
4	29	191	220
Overall	997	1,441	2,438

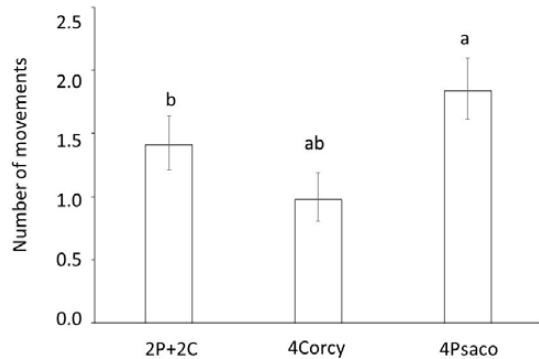


Fig. 7. Mean number of movements made by individual foundresses within replicates according to group foundress treatment (“4Psaco” = 4 females developed on the same *Psacotha hiliaris hiliaris*; “4Corcy” = 4 females developed on the same *Corcyra cephalonica*; “2P+2C” = 2 females developed on a *P. h. hiliaris* plus 2 developed on a *C. cephalonica*). The standard errors around the means are asymmetric due to back-transformation from log-scale estimates. Significant differences are indicated by different letters (Tukey post hoc test).

Discussion

The present research provides information about the reproductive behavior and performance of *S. brevicornis* according to the species of host that the wasps developed on and the species of hosts they subsequently encounter. The longer-term consequences of rearing *S. brevicornis* on *C. cephalonica* were not assessed in previous studies, and thus there was no information on the likely effectiveness of female *S. brevicornis* reared on this host. The current study has begun to address this knowledge gap, albeit within the laboratory environment. However, laboratory assays can be useful to predict the field performance of mass-released parasitoids and to attune release rates accordingly (Bourchier et al. 1993).

We found that the death of foundress females was a common occurrence and that dead females were bitten into 2 parts. Although foundresses may occasionally kill other potential foundresses by biting them into two (as reported in *S. guani*, Guo et al. 2023), it is common across several *Sclerodermus*–host associations that death occurs as a result of defense by attacked hosts (summarized in Abdi et al. 2020a). It seems most likely that the deaths we observed were principally due to the actions of hosts, rather than of other foundresses, as, in the choice test experiment, no foundress deaths occurred after hosts were paralyzed. The no-choice tests (presentation of a single “natural” host) indicated that the host that foundresses developed on did not influence the probability of a female being killed. In the choice test, we similarly found no difference in mortality between groups of foundresses that all originated from the natural host or all from the factitious host. Although these results suggest that there is no host-of-origin effect on the ability of *S. brevicornis* females to tackle *P. b. hiliaris* larvae, in the choice

test we also found that foundress mortality was highest when the females in a group originated from 2 different hosts and the form of Fig. 3 suggests that developing on *C. cephalonica* might be a disadvantage in future host attack. Another candidate explanation is that females with different origins recognized each other as nonsiblings and exhibited heightened interfemale aggression (as reported in *S. guani*, Guo et al. 2023), but, in terms of explaining high mortality, this runs counter to the possibility that foundresses in less closely related groups are less likely to take risks in host attack (as previously reported in *S. brevicornis*, Abdi et al. 2020a, 2020b). The propensity to take risks can be reflected in the timing of host attack (Abdi et al. 2020a, 2020b), and our data indicated no effects of host-of-origin on the timing taken to attack and suppress hosts or on the time to oviposition. This not only suggests that higher mortality among mixed-origin groups is not due to kin-correlated risk-taking in host attack but also indicates that females are equally able to recognize *P. b. hiliaris* larvae as hosts, and may be equally able to suppress them, whether or not they developed on a *P. b. hiliaris* host themselves. These are key considerations in the use of factitious hosts for the production of biocontrol agents for field release.

Although foundresses took the same time to attack and oviposit on hosts of either species, the production of offspring on *P. b. hiliaris* larvae in the no choice tests was considerably lower when foundresses had developed on *C. cephalonica*. This suggests that *C. cephalonica* hosts do not provide the same quality or quantity of nutritional resources as *P. b. hiliaris*, leading to adults that develop on *C. cephalonica* having fewer teneral reserves (those available on maturation as adults), and in consequence a lower capacity to mature eggs. In the choice tests, we, in contrast, found no effect of host-of-origin on the numbers of eggs laid or the number of adult offspring ultimately produced. Clutch sizes (and, ultimately, brood sizes) were larger on *P. b. hiliaris* than on *C. cephalonica* hosts (and brood sizes on *C. cephalonica* were broadly similar to those reported by Abdi et al. 2021). As *P. b. hiliaris* larvae were an order of magnitude larger than the *C. cephalonica* provided, a difference in resource quantity coupled with clutch size adjustment (Hardy et al. 1992, Visser 1994, Zaviezo and Mills 2000, Haeckermann et al. 2007, Tang et al. 2014) provides a straightforward candidate explanation. Resource quality (biochemical/metabolomic composition) may also have differed, given the different diets of the 2 host species and that they belong to different insect orders. As well as the size of broods produced in parasitoid mass-rearing systems, the sex ratios of broods can be an important consideration for biocontrol potential: female bias is generally a positive attribute because only female parasitoids suppress hosts post-release (Ode and Hardy 2008). We found that sex ratios were extremely female biased. Although sex ratios varied according to brood size, the effect was not strong (as previously observed in *S. brevicornis* and many other members of the genus; Tang et al. 2014, Abdi et al. 2020a, 2020b, Guo et al. 2022, Lehtonen et al. 2023, Malabusini et al. unpublished data).

The distribution of foundresses across hosts indicated a clear preference for *P. b. hiliaris*, and this was not influenced by the host species that foundresses had developed on. This is an encouraging result in terms of the use of factitious hosts for the production of field-effective biocontrol agents. We further observed that *S. brevicornis* commonly moved between hosts during the entire monitoring period. This may indicate that in nature foundresses females do not always remain with their own broods until the offspring complete development. Movements may be influenced by foundresses considering the availability or quality of resources provided by their current host and/or agonistic interactions between females (Guo et

al. 2023). Our data also suggest that sibling females tend to avoid each other rather than tend a brood together on a common host: further experiments are needed to tease apart factors that influence sibling-sibling interactions and foundress group formation in relation to expectations from kin selection theory (e.g., Thompson et al. 2017). In addition, females that had emerged from *P. b. hilaris* hosts moved more frequently between hosts. One possibility is that *P. b. hilaris* hosts are larger than *C. cephalonica* and wasps developing from these have a greater physical capacity for movement, for instance due to being larger or having greater energy reserves (Gao et al. 2016, Wang and Keller 2020).

In conclusion, prior studies have shown that *S. brevicornis* can be reared on *C. cephalonica*, but the subsequent performance and preferences of adults reared from this factitious host species were not evaluated. The current study suggests that *S. brevicornis* reared from *C. cephalonica* do have some disadvantages compared with those that have developed on *P. b. hilaris* but also that they nonetheless recognize, and indeed have a preference for, *P. b. hilaris*, which they are then able to produce offspring from. From the point of view of biocontrol, the ability to develop on a factitious host is convenient and a subsequent preference for, and ability to utilize, the target pest as a host is highly desirable.

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Author Contributions

Serena Malabusini (Conceptualization-Equal, Data curation-Equal, Formal analysis-Equal, Investigation-Equal, Methodology-Equal, Writing – original draft-Equal), Ian Hardy (Data curation-Equal, Formal analysis-Equal, Writing – original draft-Equal), Costanza Jucker (Funding acquisition-Equal, Investigation-Equal, Project administration-Equal, Writing – review & editing-Equal), Greta Guanzani (Investigation-Equal), Sara Savoldelli (Investigation-Equal, Writing – review & editing-Equal), Daniela Lupi (Conceptualization-Equal, Methodology-Equal, Project administration-Equal, Resources-Equal, Supervision-Equal, Writing – original draft-Equal)

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