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Reproductive biology of *Holepyris sylvanidis* (Hymenoptera: Bethylidae)

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1   **Reproductive biology of *Holepyris sylvanidis* (Hymenoptera: Bethylidae)**

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24   **ABSTRACT**

25   *Holepyris sylvanidis* (Hymenoptera: Bethylidae) is reported as a cosmopolitan parasitoid of

26   coleopteran including a major pest of stored products, the confused flour beetle *Tribolium*

27   *confusum*. The reproductive biology, and hence biocontrol potential of *H. sylvanidis* is

28   relatively little known. Here we describe and evaluate aspects of egg size, immature

29   development, adult mating behaviour, pre-oviposition time, fecundity, host attack and use,

30   and adult longevity. Our key findings are that ≈72% of presented *T. confusum* immatures

31   presented to females were attacked and ≈39% of these were fed on destructively with the

32   remainder oviposited onto. First instar host larvae and host pupae were only utilized for

33   feeding, second and third instar larvae were commonly used for either feeding or oviposition

34   while fourth, fifth and sixth instar hosts were almost exclusively used for oviposition. Females

35   usually laid single egg clutches and their average lifetime fecundity was ≈16.5 eggs. Females

36   regularly provisioned with hosts often ceased laying eggs up to several weeks before their

37   death but continued to kill and feed on hosts during this period. Adult longevity was greatly

38   enhanced by the provision of hosts (females) or by non-host food sources (males). We

39   conclude that the inoculative biocontrol potential of *H. sylvanidis* is likely to be limited by a

40   low realized fecundity and the combination with other control methods in an IPM-approach is

41   needed. Its ability to find and kill hosts may make it more suitable for inundative deployment.

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1. Introduction

There are over two thousand described species of wasps that belong to more than 100 genera within the hymenopteran family Bethylidae (Gordh and Móczár, 1990). Typically these are parasitoids of coleopteran and lepidopteran larvae (Evans, 1978; Mayhew and Hardy, 1998), although a minority of bethylid species attack hosts in the pupal stage, and some can develop on hosts belonging to other insect orders (Pérez-Lachaud et al., 2004; Zhang et al., 1984). Bethylid species have been used in empirical investigations of factors thought to influence the evolution of key behavioural and reproductive traits, such as clutch size, sex allocation, aggression and sociality (Green et al., 1982; Griffiths and Godfray, 1988; Hardy et al., 2013; Hu et al., 2012; Khidr et al., 2013; Lizé et al., 2012; Mayhew and Godfray, 1997; Mayhew and Hardy, 1998; Morgan and Cook, 1994; Suma et al., 2014; Tang et al., 2014). As the hosts of many bethylid species are pests of field-crops, stored products and in agro-forestry, bethylids have also been deployed as biological control agents in a wide range of agro-ecosystems (Conlong and Graham, 1998; Damon and Valle, 2002; Gothilf and Mazor, 1987; Legner and Gordh, 1992; Jiang et al., 2015; Legner and Silveira-Guido, 1983; Pérez-Lachaud et al., 2004; Sreenivas and Hardy, 2016; Venkatesan et al., 2007; Yang et al., 2014). Most basic and applied studies of bethylids have, however, focused on species in a small sub-set of genera, such as *Sclerodermus* Latreille and *Cephalonomia* Westwood in the sub-family Epyrinae and *Goniozus* Förster in the sub-family Bethylinae. Here we contribute to knowledge of the reproductive biology of *Holepryis sylvanidis* (Brèthes) (= *Parepypris sylvanidis* Brèthes, *Rhabdepyris zeae* Turner and Waterston); this a little studied species in a relatively unstudied epyrine genus and a beneficial natural enemy of stored products pests.

Holepyris sylvanidis is reported as a cosmopolitan parasitoid of coleopterans in the families Cucujidae, Curculionidae and Tenebrionidae (Abdella et al., 1985; Gahan, 1930; Evans, 1978; Gordh and Móczár, 1990; Eliopoulos et al., 2002; O'Connor and Ronayne,

3

73   2002). Among the Tenebrionidae the preferred host is the confused flour beetle *Tribolium*

74   *confusum* Jacquelin du Val (Ahmed and Islam, 1988; Hagstrum et al., 2012; Lorenz et al.,

75   2010), which feeds on many kinds of flour, grains, cereal products, dry fruits and nuts (Park,

76   1934; Sutton et al., 2011). *Tribolium confusum* infests in flour mills, feed mills, warehouses,

77   boxcars, semolina mills peanut shelling plants, bakeries, grocery stores, pet stores and human

78   residences (Eliopoulos et al., 2002; Hagstrum et al., 2012; Hagstrum and Subramanyam,

79   2009; Prozell and Schöller, 1998), particularly in areas with Mediterranean climate

80   (Trematerra et al., 2007). Contamination by *T. confusum* of flour mills, and other facilities,

81   can have significant economic consequences, including costs associated with treatment and

82   monitoring, rejection and return of contaminated products and loss of consumers' trust

83   (Campbell and Hagstrum, 2004). Application of synthetic pesticides may reduce *T. confusum*

84   populations but may have undesirable side effects, and is not applicable in organic bakeries

85   and mills (Lorenz et al., 2010; Prozell and Schöller, 1998). Knowledge of *T. confusum* natural

86   enemies could thus lead to economic benefits.

87   Previous studies of the biology of *H. sylvanidis* have described its mating behaviour,

88   foraging behaviour and host attack (Ahmed et al., 1997; Ahmed and Islam, 1988; Fürstenau et

89   al., 2016; Lorenz et al., 2010), while other studies have recorded its associations with pest

90   species and other natural enemy species in stored-product agroecosystems (Adler et al., 2012;

91   Eliopoulos et al., 2002). Foraging *H. sylvanidis* females are attracted by odours emanating

92   from the faeces of *T. confusum* larvae (Fürstenau et al., 2016). Lorenz et al. (2010) reported

93   that females are able to penetrate 8 cm into the flour substrate to find *T. confusum*, but that the

94   success in finding hosts declined with depth and also as particle size decreased. Hosts that are

95   attacked are typically transported to a crevice or hole prior to oviposition (Ahmed et al., 1997;

96   Lorenz et al., 2010). *Holepyris sylvanidis* females attack and feed on *T. confusum* larvae in the

97   early instars and oviposit on larger larvae, normally laying a single egg onto each hosts, but 2

98   and 3 eggs clutches are occasionally observed (Ahmed et al., 1997). Notwithstanding these

4

99 prior studies, obtaining empirical information on *H. sylvanidis* has been hampered by

100 difficulties associated with collecting and then rearing this species under laboratory

101 conditions. In this paper we report a viable laboratory-rearing protocol for *H. sylvanidis* along

102 with observations on its behaviour and reproduction. Specifically, we describe and evaluate

103 aspects of egg size, immature development, adult mating behaviour, pre-oviposition time,

104 fecundity, host attack and use, and adult longevity. We discuss these new findings in regard to

105 both the general biology of bethylid wasps and the potential for *H. sylvanidis* to suppress

106 populations of pests.

107

108 **2. Materials and methods**

109 *2.1 Host and parasitoid cultures*

110 The host *T. confusum*, was reared in 12 cm diameter Petri dishes containing 20 g of

111 *Triticum aestivum* flour and 5% of brewers' yeast as a substrate (Athanassiou and

112 Kavallieratos, 2014). The *H. sylvanidis* used were a strain collected from wheat infested by *T.*

113 *confusum* in a grain centre warehouse at Enna (Italy). Parasitoids were reared by keeping two

114 female and one male *H. sylvanidis* in a 12 cm diameter Petri dish with fourth instar host

115 larvae (Ahmed et al., 1997), with fresh fourth instar larvae added each week. Two squares (2

116 cm × 2 cm) of corrugated cardboard were placed into each Petri dish to provide places for

117 female parasitoids to hide paralysed hosts (Ahmed and Islam, 1988). Adult *H. sylvanidis* host

118 feed (Ahmed et al., 1997) but, in order to enhance egg production, were provided with a

119 supplementary diet composed of water (1 ml), honey (0.1 g), pollen (7 grains) and royal jelly

120 (0.05 g) absorbed onto a 1×1cm piece of filter paper. All cultures and experiments were

121 maintained at 27±1°C and 65±5 % relative humidity.

122

123 *2.2 Life history evaluations*

124   We evaluated aspects of egg size, immature development, adult mating behaviour, pre-

125   oviposition time, fecundity, host use and adult longevity.

126

127   *2.3 Development of offspring*

128   Parasitoid cultures were inspected daily and any fourth instar host larvae bearing a *H.*

129   *sylvanidis* egg were collected kept individually in 4 cm diameter Petri dishes. The length and

130   width of each egg was measured under a binocular dissecting microscope with image analysis

131   software. The development of each *H. sylvanidis* offspring was observed daily until adult

132   eclosion. Replicates in which the egg did not mature to adulthood were excluded. We

133   recorded the number of days before the egg hatched (when head of the larva emerged from

134   the chorion, according to Powel 1938), the durations of the larval and pupal stages and the sex

135   of the emerging adult. We also recoded the time between each egg hatching and the

136   penetration of the body of the host by the larva, the time taken for the host to be consumed

137   and the time it took for larvae to spin a silken cocoon and pupate.

138

139   *2.4 Mating behaviour*

140   In each replicate, one male and one female *H. sylvanidis*, with no prior mating experience

141   and within one day of eclosion as adults, were placed into a 4 cm diameter Petri dish and

142   observed. In order to ensure that the adults were unmated, their cocoons had been isolated

143   from the cultures for at least 4 days before adult emergence. Courtship and mating

144   interactions were observed and the time until mating (pre-copulatory time) and the duration of

145   copulation, from when the male mounted the female until the male dismounted, was recorded.

146   After each replication, the Petri dish was cleaned with ethanol to remove any chemical

147   stimuli.

6

148 *2.5 Pre-oviposition period*

149   In each replicate, a newly emerged (<1 day old) and unmated female was placed into a 12

150 cm diameter Petri dish containing a square of corrugated cardboard and 5 fourth instar *T.*

151 *confusum* host larvae, which are the preferred host developmental stage (Ahmed et al., 1997).

152 In some replicates a newly emerged male *H. sylvanidis* was also present, leading to the female

153 being mated. The hosts were observed daily until the first *H. sylvanidis* egg was observed.

154 Host larvae that were fed upon or had died and were no longer suitable for oviposition were

155 replaced at each observation. The pre-oviposition period was calculated as the number of days

156 between the emergence of the female and the first egg laid (Finlayson, 1950). There were 50

157 replicates using unmated and 50 using mated females.

158

159 *2.6 Host attack and use*

160   In each replicate ($n = 10$) one ≤5 day-old female was placed into a 12 cm diameter Petri

161 dish containing 35 hosts, comprising 5 hosts of each of the 6 larval instars and 5 pupae. Host

162 attack behaviour was observed. Each replicate was inspected daily for 10 days noting the

163 number of hosts that had been fed upon or paralysed by the female. We note that it is difficult

164 to detect the paralysis of host pupae but feeding damage was straightforward to observe.

165

166 *2.7 Lifetime fecundity*

167   In each replicate, a newly emerged female was placed into a 12 cm diameter Petri dish

168 containing a square of corrugated cardboard and supplied with fourth instar host larvae twice

169 per week: on Mondays, 5 fresh hosts were added to the Petri dish and on Fridays all larvae

170 were removed and 10 fresh hosts provided. Hosts with eggs laid on them were collected on

171 both Mondays and Fridays. The procedure was repeated until each female died. In some

172   replicates a newly emerged male *H. sylvanidis* was also present from the start such that the

173   female was mated.

174

175   *2.8 Longevity*

176   We evaluated the longevity of females ($n = 92$), with and without the provision of hosts

177   and of males, with and without the provision of food (while adult males may not interact with

178   hosts they could potentially feed on nectar and pollen). Mated females (<1 day old) were

179   placed individually into plastic vials (2.6 cm × 0.6 cm) stoppered with cotton gauze. In some

180   replicates, 5 fresh host larvae were provided twice per week and any previously provided

181   hosts removed. In other replicates the females were starved. Male longevity was similarly

182   evaluated ($n = 144$); males were either starved or provided with food on the gauze stopper

183   twice per week. Some fed males received sucrose solution (0.5 g per 2.5 ml water) and others

184   were provided with sucrose solution with 4 grains of pollen added. For both male and

185   females, longevity was evaluated by inspecting the vial each day until the wasp died.

186

187   **3. Statistical analysis**

188   Where possible data were explored using generalized linear modelling (log-linear

189   modelling, and parametric survival analysis; Crawley, 1993) but non-parametric Mann-

190   Whitney U tests were employed when error distributions did not conform well to parametric

191   assumptions. All tests were two-tailed and used the 0.05 significance level. Data were

192   analysed using GenStat (version 17, VSN International, Hemel Hempsted) and StatPlus

193   (version 6.0.3, AnalystSoft Inc.).

194

195   **4. Results**

8

196 *4.1 Development of offspring*

197   Eggs were on average 0.32 (±0.003 SE) mm long and 0.13±0.010 mm wide (*n* = 50). Eggs
198 typically hatched after 2 days and there was no significant difference in time to hatching
199 between eggs that eventually developed into males and eggs that developed into females
200 (Table 1). Hatched larvae inserted the mouthparts into the host body to feed and initially fed
201 on the host completely ectoparasitically but after around 3.5 days each larva penetrated the
202 host such that its anterior half was embedded into the host. Each larva fed on its host until
203 only the empty exoskeleton remained, around 2-4 days after penetrating the host. For both
204 sexes, the larval stage typically lasted nearly 9 days (Table 1). It was not possible to document
205 the number of larval instars because no exuviae were observed (as in other epyrines,
206 Finlayson, 1950; Mertins, 1980). Once hosts were consumed, each larva spun a white cocoon
207 over a 1-2 day period before pupating. In some cases a proper cocoon was not formed but
208 pupae that were not fully enclosed in cocoons were able to develop to adulthood. *H.*
209 *sylvanidis* pupae are initially white but become dark. The large compound eyes start to
210 become brownish by the end of the first day of pupation and become darkly pigmented by the
211 second day. The thorax becomes dark before the abdomen and the head. The intersegmental
212 membrane of the abdomen is the last part to change colour, becoming completely dark one
213 day after the adult wasp emerged. The pupal stage of males was more than a day shorter than
214 females (protandry) (Table 1) and on emergence a typical meconium (Rilett, 1949) was
215 observed. Adult males are smaller than adult females.

216

217 *4.2 Mating behaviour*

218   We identified multiple components of courtship and mating behaviour (Table 2) and the
219 sequence in which they typically occurred (Fig. 2). Courtship started when the male verified
220 the opportunity to mate by approaching the female and touching her with his antennae.

9

221 Unreceptive females moved away from the male rapidly but receptive females vibrated their
222 antennae then turned their bodies by 180°. Then the wasps touched antennae from one to
223 seven times, in separate bouts, prior to mating. Males then suddenly jumped onto the female
224 and copulation occurs. On occasion, when a female was receptive but the male did not initiate
225 courtship, the female touched the male's abdomen from behind with the antennae, apparently
226 to stimulate the male to enter courtship.

227 Copulation began after a mean courtship period of 208.6 ± 30.2 seconds (n = 30) and the
228 mean duration of copulation was 119.5 ± 8.2 s. During copulation the male gripped the female
229 with his body at 90° to hers, and the antennae of both sexes were motionless. Usually the
230 female did not move during copulation but in some instances she ran around. Male assumed
231 an elevated position and displayed rhythmic movements of their legs during copulation, but
232 only when the female was stationary. At the end of the copulation, the males dismounted from
233 the female.

234

235 *4.3 Pre-oviposition period*

236 Mated females began laying eggs after 3.68 ± 0.28 days. Unmated females had a
237 significantly longer pre-oviposition period (5.36 ± 0.51 days; Mann-Whitney U-test, U =
238 927.5, P = 0.024, Fig. 2).

239

240 *4.4 Host attack and use*

241 When a female *H. sylvanidis* encountered a potential host she inspected it by touching its
242 surface with her labial and maxillary palps. On recognizing a larva as a suitable host, the
243 female immediately attacked it by grasping it with her legs and bending her abdomen around
244 the larva and rotating her body by 360 degrees for three or four times. The attack is very rapid
245 and it is difficult to observe exactly when the female stings the host with venom. The host was

10

246 typically paralysed immediately and permanently (on occasion the host larva later regained

247 activity and began to crawl, with a parasitoid egg adhering to its venter). Females continued to

248 touch the host with their antennae for some seconds after stinging. While paralysed hosts were

249 almost motionless they showed rhythmic movements of their legs. Sometimes the female

250 attacked the legs of the host larva, biting the intersegmental membrane. Although each host

251 larva was entirely explored, females paid most attention to the segments close to the legs. The

252 females then typically grasped their host with their mandibles and moved it to another locality

253 prior to oviposition, but some hosts were instead fed upon.

254 Of all hosts presented, 72.3% were utilized for feeding or were parasitized by *H. sylvanidis*

255 females; of these, 39.1% were fed upon. Host acceptance and usage differed according to host

256 developmental stage. Host acceptance increased as larvae had developed until the fifth instar

257 and then declined (Fig. 4). In terms of usage, first instar larvae and pupae were only utilized

258 for feeding, second and third instar larvae were commonly used for either feeding or

259 oviposition while fourth, fifth and sixth instar hosts were almost exclusively use for

260 oviposition (Fig. 3). As far as we could discern, each host was either fed upon or oviposited

261 upon, but not both.

262 Ovipositing females usually laid a single egg onto the host, but clutches of two eggs were

263 occasionally laid. Eggs were normally placed longitudinally between the first and second

264 abdominal sternite, as figured in Reichmuth et al. (2007), but sometimes the egg was laid on

265 the second and third sternite, or between the third and fourth abdominal sternites.

266

267 *4.5 Lifetime fecundity*

268 Females laid on average 16.42 (+SE = 0.871, -SE = 0.828) eggs during their lifetimes (*n* =

269 68). There was no significant difference in the overall fecundity realized by mated and

270 unmated females (log-linear ANOVA corrected for overdispersion by assuming a quasi-

11

271 Poisson distribution of errors using an empirically estimated scale parameter: $F_{1,66} = 2.42$, $P = $

272 0.124, deviance explained = 3.5%). Egg laying activity was initially higher among mated

273 females than unmated females and declined as females aged, with a more gradual decline

274 among unmated females (Fig. 4a). The risk of a female dying increased between successive

275 observation periods (fitting a Weibull model of cohort survival reduced the deviance

276 significantly compared to an exponential model, $G_1 = 91.1$, $P < 0.001$) and unmated females

277 lived significantly longer than mated females ($G_1 = 25.6$, $P < 0.001$, Fig. 4b). In many

278 replicates females ceased laying eggs before all of the provided hosts had been oviposited on

279 and several weeks before their time of death. The period of active reproduction was

280 significantly shorter among mated females than among unmated females ($G_1 = 14.98$,

281 $P<0.001$) but we observed that females continued to kill and eat hosts after ceasing to

282 oviposit. The functions describing the models for mated and unmated females are

283 respectively: = initial number × (Exp(-(0.0001219 × Observation period$^{3.684}$)) and = initial

284 number × (Exp(-(0.00003438 × Observation period$^{3.684}$)).

285

286 *4.6 Longevity*

287 Female longevity was significantly greater when females were provided with fourth instar

288 *T. confusum* larvae (48.6 ± 2.7 days) then when they were not (8.1 ± 0.5 days) (Weibull

289 cohort survival model: $G_1 = 355.16$, $P < 0.001$): all unprovisioned females died within 20

290 days while females with hosts lived up to 86 days (Fig. 5a). Similarly, male longevity was

291 affected by feeding treatment ($G_2 = 83.40$, $P < 0.001$, Fig. 5b): when starved males lived

292 around 7 (± 0.2) days, males provided with sucrose lived on average for 18.18 (± 1.64) days

293 and the males nourished with the sucrose solution and pollen lived on average for 35.48 (±

294 4.97) days. Although the survival of males fed with sucrose only and sucrose and pollen was

295 initially similar (Fig. 5b), males fed with sucrose and pollen had significantly greater

12

296   longevities ($G_2 = 21.10$, $P < 0.001$). The functions describing the models for the longevity of

297   females and fed females are respectively: $= 100 \times (\text{Exp}(-(0{,}01574 \times (\text{Observation}$

298    $\text{period}^{1,928}))))$ and $= 100 \times (\text{Exp}(-(0{,}0005029 \times (\text{Observation period}^{1,928}))))$. The functions

299   describing the models for the longevity of males, males and sugar, males sugar and protein are

300   the following: $= 100 \times (\text{Exp}(-(0{,}02237 \times (\text{Observation period}^{1,928}))))$, $= 100 \times (\text{Exp}(-$

301    $(0{,}0027559 \times (\text{Observation period}^{1,928}))))$, $=100 \times (\text{Exp}(-(0{,}00058318 \times (\text{Observation}$

302    $\text{period}^{1,928}))))$.

303

304   **5. Discussion**

305   *Holepyris sylvanidis* is a natural enemy of immature beetles, acting either as a destructive

306   host feeder or as a parasitoid of each suitable host encountered. As such its observed biology

307   conforms broadly to expectations derived from knowledge of other species within the bethylid

308   sub-family Epyrinae (e.g. Lauzière et al., 1999; Mayhew and Hardy, 1998; Pérez-Lachaud

309   and Hardy, 1999). Further, host hiding prior to oviposition is exhibited in common with

310   several other Epyrinae (Eliopoulos et al., in press; Griffiths and Godfray, 1988) as is laying

311   single-egg, or otherwise, small clutches (Ahmed et al., 1997; Finlayson, 1950; Mayhew and

312   Godfray, 1997; Mayhew and Hardy, 1998). The observed positions in which eggs were

313   observed to be laid onto the hosts agree with patterns reported by Ahmed and Islam (1988).

314   *Holepyris sylvanidis* is protandrous, with males emerging in advance of females and

315   mating occurs at the natal site. This observation agrees with Abdella et al. (1985). We

316   observed that mating occurred soon after emergence and that males jump suddenly onto the

317   female just prior to copulation, both observations tally with prior reports (Ahmed et al., 1997;

318   Ahmed and Islam, 1988). Ahmed et al. (1997) further reported that females remain stationary

319   during copulation, while we observed some females running around whilst *in copula*. The

320 mating behaviour of *H. sylvanidis* is not complex and would not present obstacles to this

321 species being mass reared for release as a biocontrol agent.

322   *Holepyris sylvanidis* is synovigenic (Ahmed et al., 1997) and host feeding likely serves to

323 supply nutrients for egg maturation, as in other epyrine species (Lauzière et al., 1999; Pérez-

324 Lachaud and Hardy, 1999). We observed that female *H. sylvanidis* fed primarily on host

325 haemolymph but were also able to eat the tissues of the host larvae. Ahmed et al. (1997) have

326 previously reported that *H. sylvanidis* females attack and feed on the first to the fourth larval

327 instars but not the fifth and sixth, or the pupal stage. In contrast, we observed some feeding on

328 all host developmental stages, although it was rare on fourth, fifth and sixth instar larvae

329 which are primarily used for oviposition. The major discrepancy between our results and

330 those of Ahmed et al. (1997) is that they reported an absence of host feeding on pupal *T.

331 confusum* while we observed this commonly.

332   While host feeding leads to more hosts being killed by a current generation of adult

333 parasitoids, it reduces the number of offspring produced per host found and as such may not

334 be a desirable characteristic of beneficials as destructive host-feeders are predicted to suppress

335 host population equilibria as less strongly than other parasitoids (Emerick and Singh, 2016;

336 Jervis et al., 1996a, b; Jervis and Kidd, 1986). Host feeding could influence both the pre-

337 oviposition period and the time required for laying successive eggs once a female has started

338 to oviposit. For example, in the epyrine *Cephalonomia stephanoderis* Betrem the pre-

339 oviposition period was shorter when there were more hosts available (Lauzière et al., 1999).

340 As in several other epyrine species (Mayhew and Heitmans, 2000; Yamasaki, 1982) unmated

341 female *H. sylvanidis* had longer pre-oviposition periods than mated females, possibly because

342 there are fitness advantages to being able to produce both male and female offspring and thus

343 virgin females may tend to delay the production of progeny to increase the probability of

344 being mated before they oviposit (Godfray, 1990; Guertin et al., 1996).

14

345   We observed that fourth, fifth and sixth instar host larvae were usually used for

346   oviposition, whereas Ahmed et al. (1997) found that only fourth instar larvae were oviposited

347   on. It is likely that early instar hosts are too small to provide sufficient nutrients to support

348   parasitoid development and are thus fed on (see above) but why no oviposition was observed

349   on larger, fifth and sixth instar, hosts by Ahmed et al. (1997) is unclear. The fact that

350   oviposition onto pupal *T. confusum* was observed by neither study suggests that biochemical

351   changes during pupation make pupae nutritionally unsuitable for parasitoid development. Key

352   nutritional differences between the developmental stages of *T. confusum* could potentially be

353   identified using a metabolomics approach tailored to low biomass organisms (Kapranas et al.,

354   submitted; Snart et al., 2015) and the same approach could also be employed to understand

355   better the range of host species it attacks (Eliopoulos et al., 2002).

356   The realized fecundity (a mean of 16.4 eggs laid by each female) of *H. sylvanidis* female is

357   lower than estimates for other epyrines which are natural enemies of stored products pests:

358   *Cephalonomia waterstoni* Gahan, 102 eggs (Finlayson, 1950) and *C. tarsalis*, 85 eggs

359   (Powell, 1938) or 50-200 eggs (Lukáš, 2007). Unlike *C. tarsalis* (Cheng et al., 2003), mating

360   status did not affect fecundity but abiotic environmental conditions could have influenced our

361   estimate of fecundity, given that the epyrine *Prorops nasuta* Waterston is recorded as laying

362   322 eggs at 27°C and only 33 at 18°C (Infante, 2000). However, Abdella et al. (1985) found

363   in a laboratory study optimum conditions for *H. sylvanidis* of 27°C and 57.7% relative

364   humidity. This is close to the conditions in our study. We further found that *H. sylvanidis*

365   females become egg limited as they age and thus do not oviposit on all suitable hosts

366   provided. Some mated females ceased laying as much as 1-2 weeks before dying while in

367   some unmated females oviposition ceased as many as 4 weeks before death.

368   The provision of hosts clearly affected female *H. sylvanidis* longevity, probably because

369   provisioned females were able to host-feed to obtain nutrients for somatic maintenance, as

15

370 well as egg production, extending their adult life expectancies from around 8 to around 48

371 days. Similarly, estimates of longevity of females of species in the epyrine genus

372 *Cephalonomia* provided with hosts range between 30 and 94 days (Finlayson, 1950; Pérez-

373 Lachaud and Hardy, 1999; Powell, 1938; van Emden, 1931; Yamasaki, 1982), depending on

374 environmental conditions, while without hosts longevity is typically between 10-20 days

375 (Yamasaki, 1982). We also found that unmated *H. sylvanidis* lived longer than mated females,

376 in contrast to *C. tarsalis* in which both mated and unmated females lived an average of 35

377 days (Powell, 1938). Longevity can play an important role in the impact of parasitoids on pest

378 populations (Hausmann et al., 2005).

379　Male parasitoids are typically shorter-lived than females; for instance, Powell (1938)

380 reported that *C. tarsalis* males live for 6 days and our estimate of male *H. sylvanidis* longevity

381 is 7 days. However, male *H. sylvanidis* longevity was greatly enhanced by the provision of

382 nutrients. Male *H. sylvanidis* do not attack or feed on host larvae but may feed on non-host

383 sources of sugar and proteins such as nectar and pollen. We found that providing sucrose

384 alone approximately doubled male life-expectancy; similar enhancements have been reported

385 in other parasitoid species (Fadamiro and Heimpel, 2001). Further providing both sucrose and

386 pollen increased the average male *H. sylvanidis* longevity to 35 days, with some males living

387 over 150 days, possibly because sugars could be used to aid the digestion of pollen (Sigsgaard

388 et al., 2013). Further work will be required to establish how diet components influence

389 longevity and thus how parasitoids should be fed most efficiently to enhance their life-

390 histories (Hausmann et al., 2005). Fed males may also have enhanced courting, mating and

391 dispersal abilities (Kaspi and Yuval, 2000) but the relative mating abilities of fed and unfed

392 male *H. sylvanidis* have not yet been evaluated. Given that females must mate before being

393 able to produce daughters and that only females attack populations of hosts (Ode and Hardy,

394 2008), enhancing the longevity of males and their abilities to find and mate with females may

395 be beneficial in terms of improving the suppression of pest populations.

396   Although data from an industrial bakery and a mill suggest that *T. confusum* populations

397   may be regulated by *H. sylvanidis* (Adler et al., 2012; Prozell and Schöller, 1998), there is

398   limited evidence for the population dynamic interactions between *H. sylvanidis* and its hosts.

399   The need to host-feed and a relatively low fecundity are unlikely to promote *H. sylvanidis*

400   population growth and, if high parasitoid densities are reached, intra-specific mutual

401   interference interactions may further limit its efficacy for biocontrol (Eliopoulos et al., in

402   press).

403

404   **6. Conclusions**

405   *Holepyris sylvanidis* is a natural enemy of important stored product pests, most notably the

406   flour beetle *Tribolium confusum,* and has the potential to be beneficially deployed for

407   biological control, thus helping to decrease reliance on synthetic pesticides and maintain the

408   organic status of some bakeries (Lorenz et al., 2010; Prozell and Schöller, 1998). While its

409   ability to find hosts within the substrate is good (Lorenz et al., 2010), our data suggest that its

410   realized fecundity is low and on that basis it seems unlikely that this species will have the

411   capability of suppressing pest populations below an economic threshold after inoculative

412   biocontrol release, as may be the case for other epyrine species that attack stored products

413   pests (Eliopoulos et al., in press; Powell, 1938) or field crop pests (Batchelor et al., 2006).

414   Given that many encountered hosts are killed by adult females feeding upon them, and that

415   host-feeding enhances female longevity, *H. sylvanidis* may have better potential for

416   inundative or augmentative deployment, largely as a predator, but whether this is

417   economically feasible will depend on the efficiency of mass rearing methods. As shown here,

418   the food supplemented during rearing can increase the number of progeny per female, but

419   more research on mass-rearing is needed. Indeed, data from a flour mill suggest that *T.*

420   *confusum* population suppression was achieved when several hundred *H. sylvanidis* were

17

421   released every two weeks but ceased once parasitoid release stopped (Adler et al., 2012).

422   However, the last mentioned study also proved the potential for establishment of *H. sylvanidis*

423   after release. The longevity of this parasitoid makes it a good candidate for an inoculative

424   release strategy implemented in an integrated control strategy, e.g. with effective sanitation

425   programs and heat treatments (Schöller et al., 2006).

426

427   **References**

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616 **Figure captions.**

617

618 **Table 1** Development time (days ± SE) of *Holepyris sylvanidis* eggs, larvae and pupae.

619 Means within rows followed by different letters are significantly different (Mann-Whitney-U

620 test, P<0.05).

621 **Table 2.** Components of *Holepyris sylvanidis* mating behaviour

622 **Fig. 1.** Schematic representation of the course of events in a typical sequence of mating

623 behaviour. Each of the main behaviours are reported in upper case, with successive

624 component behaviours reported in lower case.

625 **Fig. 2.** Pre-oviposition period for unmated and mated *H. sylvanidis* females. * = mean

626 significantly different, Mann-Whitney U-test, P < 0.05

627 **Fig. 3.** The use of presented hosts over ten across ten replicates, with 5 hosts of each of the 7

628 stages present per replicate. The maximum number of hosts of a given stage that could be

629 attacked was 50. Bars are shown stacked.

26

630 **Fig. 4.** Egg production and longevity of females supplied with hosts twice per week until

631 death. There were two observation periods per week, such that 30 periods represents ≈105

632 days. Panel (a) shows the egg production by females alive at a given period. Panel (b) shows

633 the survival of the same cohorts of females: Data are shown as dashed lines and the models

634 fitted using Weibull survival analysis are shown as smooth curves.

635 **Fig. 5.** Cohort survival of females (a) and of males (b). Data are shown as dashed lines and

636 the models fitted using Weibull survival analysis are shown as smooth curves.

637 **Graphical abstract** Development of the parasitoid *H. sylvanidis*: the female laid and egg

638 longitudinally between the first and second abdominal sternite of the host larvae (a), the

639 parasitoid larvae developed as semi-ectoparasitoid (b), and spun a cocoon some centimetres

640 away after the host body was devoured (c,d). The overall development time changed

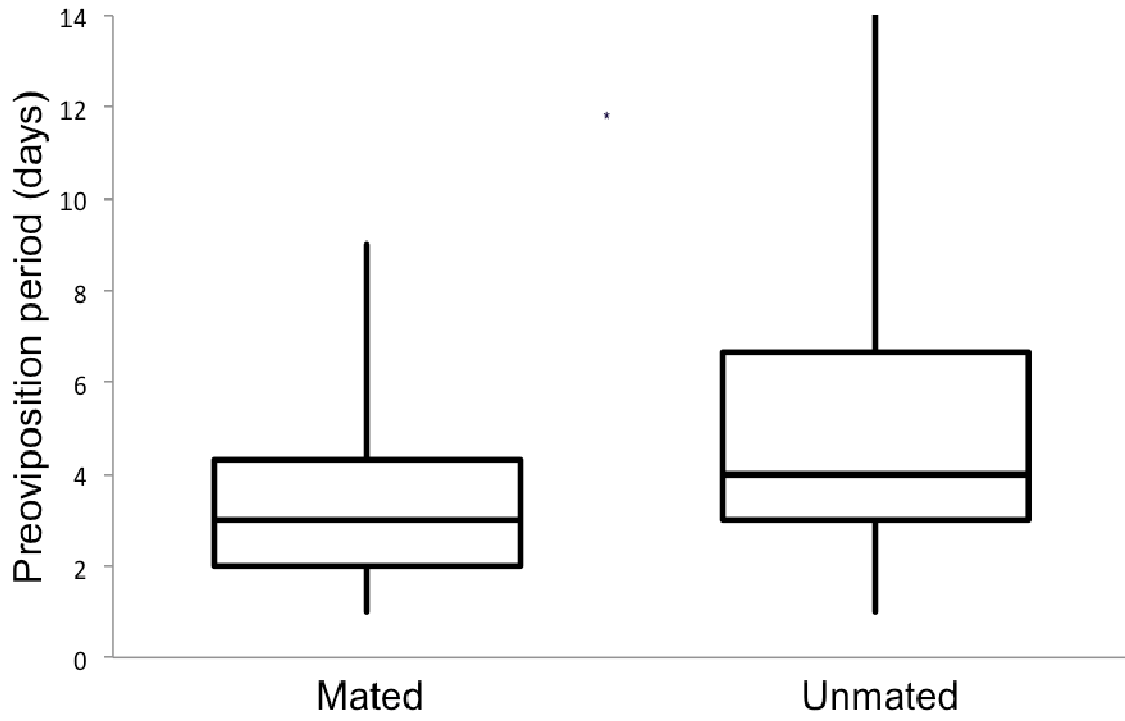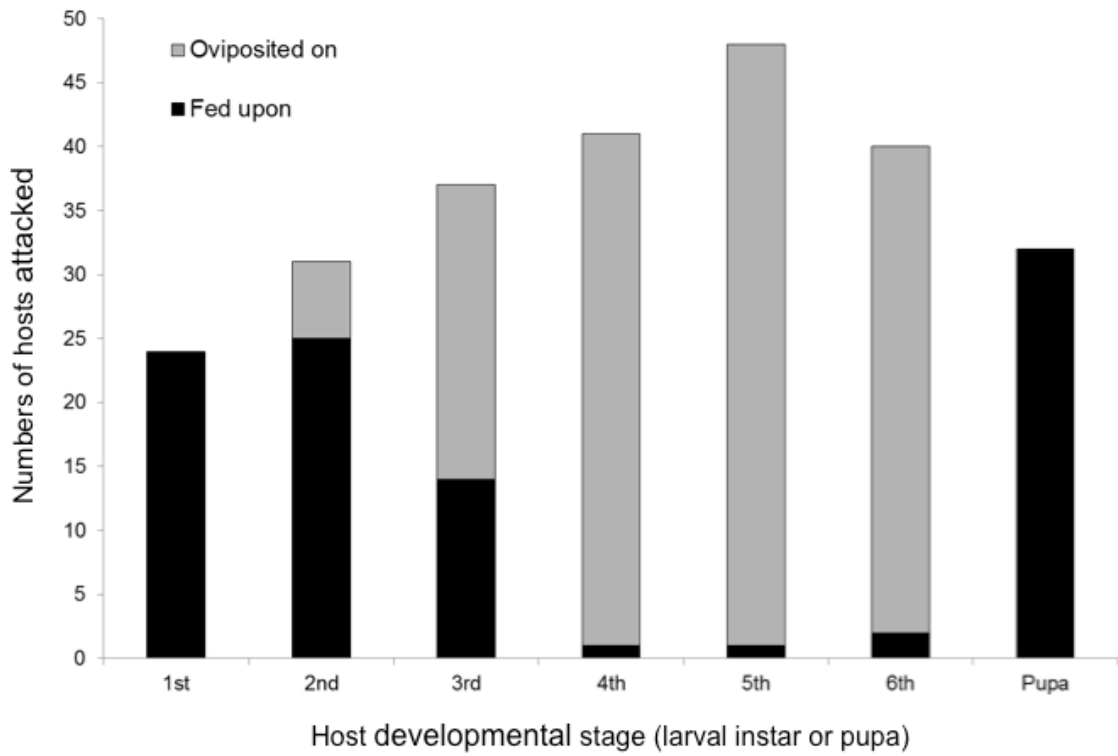641 significantly between the two sexes of *H. sylvanidis*.
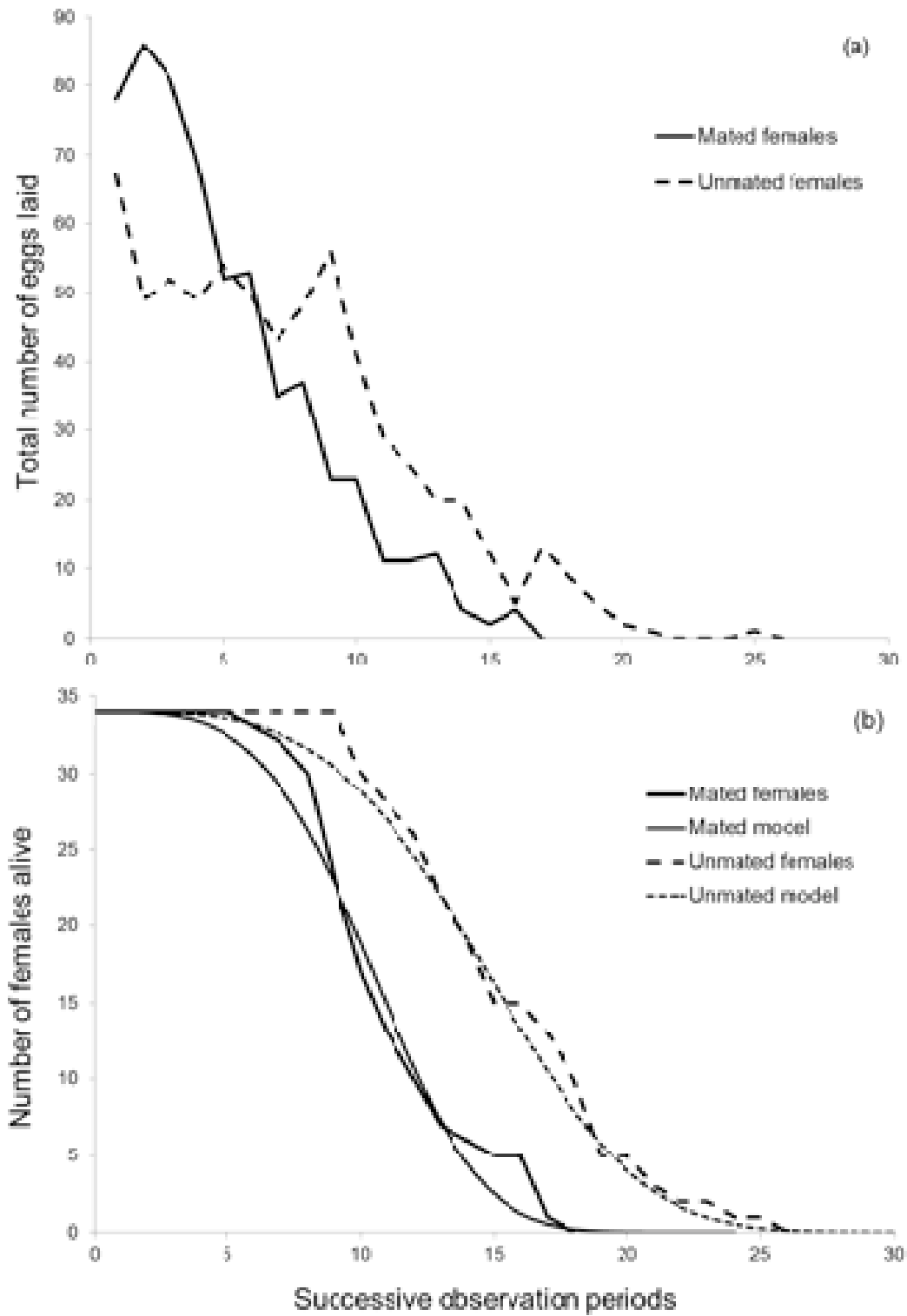
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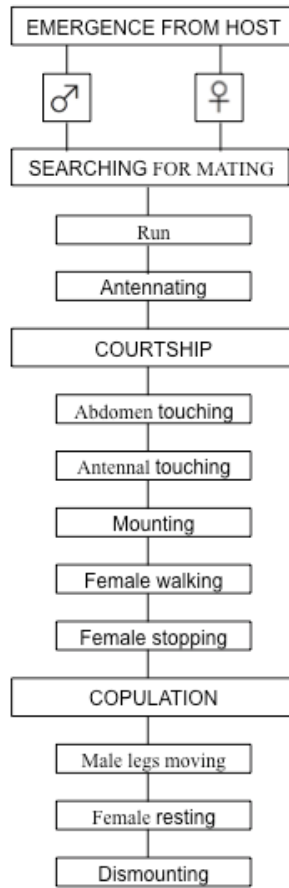
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Stage	Male (n= 35)	Female (n=35)
Egg	2.37 ± 0.11^a	2.31 ± 0.09^a
Larval	8.80 ± 0.17^a	8.86 ± 0.16^b
Pupal	11.94 ± 0.15^a	13.26 ± 0.23^b
Overall egg-to-adulthood	23.11 ± 0.21^a	24.43 ± 0.28^b

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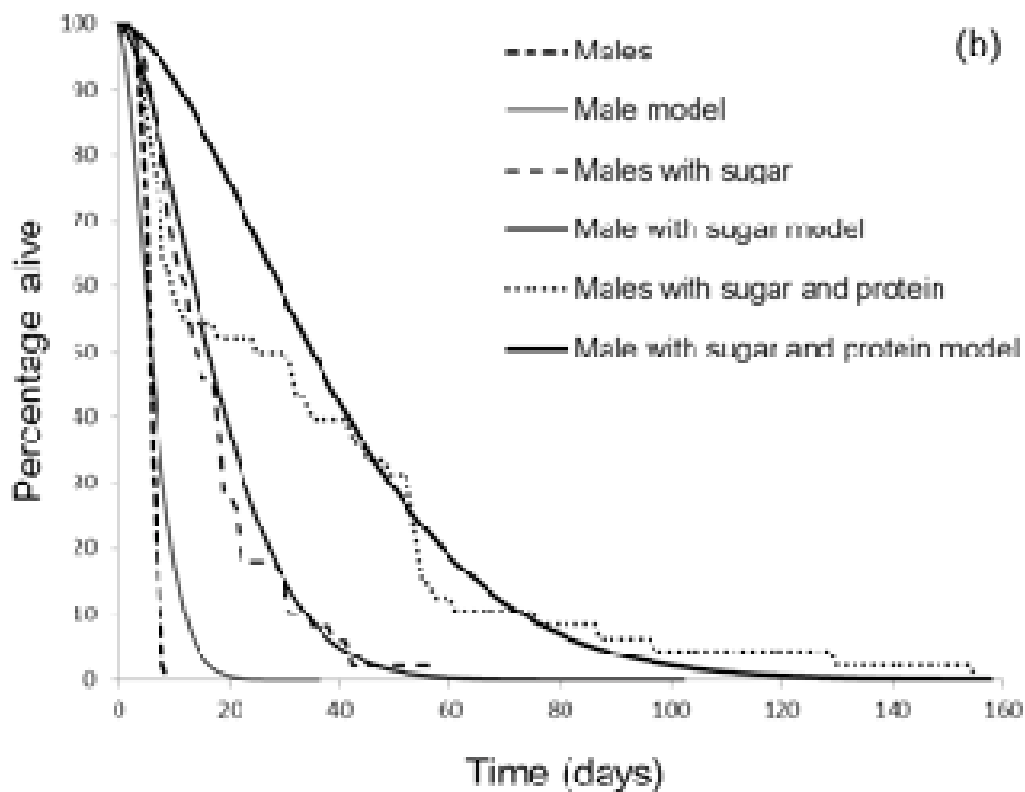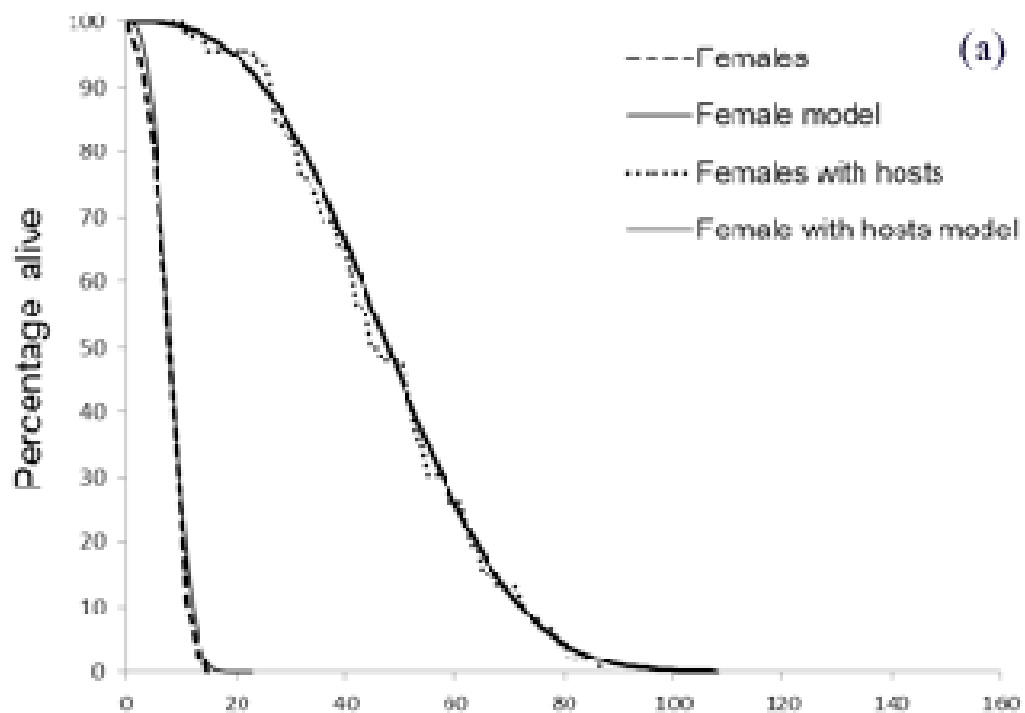
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Overall egg-to-adulthood	23.11 ± 0.21^a	24.43 ± 0.28^b

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Behaviour	Description
Run	Male and female run around
Antennating	Male and female waving the antennae kept straight while walking around
Abdomen touching	The female touches the male's abdomen with the antennae
Antennal touching	Male and female wave, and make contact with, their antennae
Mounting	The male jumps suddenly upon the female and establishes genital contact
Female walking	The female moves some centimetres, waving her antennae, while the main is still stationary and upon the female
Female stopping	The female stops walking
Copulation	Male establishes genitalia contact
Male legs moving	The male waves the legs rhythmically
Female resting	The female remains stationary during copulation
Dismounting	The male dismounts from the female and the mating ends

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661   •   The mating status significantly influences the pre-oviposition period of *H. sylvanidis*

662   •   The mating status does not influence the fecundity of *H. sylvanidis*

663   •   A food source significantly influenced the longevity of female and male

664   •   The life cycle lasts for 23 days in male and 24 days in female at 27 °C

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