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

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ABSTRACT

Holepyris sylvanidis (Hymenoptera: Bethylidae) is reported as a cosmopolitan parasitoid of coleopteran including a major pest of stored products, the confused flour beetle *Tribolium confusum*. The reproductive biology, and hence biocontrol potential of *H. sylvanidis* is relatively little known. Here we describe and evaluate aspects of egg size, immature development, adult mating behaviour, pre-oviposition time, fecundity, host attack and use, and adult longevity. Our key findings are that ≈72% of presented *T. confusum* immatures presented to females were attacked and ≈39% of these were fed on destructively with the remainder oviposited onto. First instar host larvae and host pupae were only utilized for feeding, second and third instar larvae were commonly used for either feeding or oviposition while fourth, fifth and sixth instar hosts were almost exclusively used for oviposition. Females usually laid single egg clutches and their average lifetime fecundity was ≈16.5 eggs. Females regularly provisioned with hosts often ceased laying eggs up to several weeks before their death but continued to kill and feed on hosts during this period. Adult longevity was greatly enhanced by the provision of hosts (females) or by non-host food sources (males). We conclude that the inoculative biocontrol potential of *H. sylvanidis* is likely to be limited by a low realized fecundity and the combination with other control methods in an IPM-approach is needed. Its ability to find and kill hosts may make it more suitable for inundative deployment.

Keywords: Stored product, *Tribolium confusum, Holepyris sylvanidis*, mating, fecundity, longevity.

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 *Holepryris 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, 2002). Among the Tenebrionidae the preferred host is the confused flour beetle *Tribolium confusum* Jacquelin du Val (Ahmed and Islam, 1988; Hagstrum et al., 2012; Lorenz et al., 2010), which feeds on many kinds of flour, grains, cereal products, dry fruits and nuts (Park, 1934; Sutton et al., 2011). *Tribolium confusum* infests in flour mills, feed mills, warehouses, boxcars, semolina mills peanut shelling plants, bakeries, grocery stores, pet stores and human residences (Eliopoulos et al., 2002; Hagstrum et al., 2012; Hagstrum and Subramanyam, 2009; Prozell and Schöller, 1998), particularly in areas with Mediterranean climate (Trematerra et al., 2007). Contamination by *T. confusum* of flour mills, and other facilities, can have significant economic consequences, including costs associated with treatment and monitoring, rejection and return of contaminated products and loss of consumers' trust (Campbell and Hagstrum, 2004). Application of synthetic pesticides may reduce *T. confusum* populations but may have undesirable side effects, and is not applicable in organic bakeries and mills (Lorenz et al., 2010; Prozell and Schöller, 1998). Knowledge of *T. confusum* natural enemies could thus lead to economic benefits.

Previous studies of the biology of *H. sylvanidis* have described its mating behaviour, foraging behaviour and host attack (Ahmed et al., 1997; Ahmed and Islam, 1988; Fürstenau et al., 2016; Lorenz et al., 2010), while other studies have recorded its associations with pest species and other natural enemy species in stored-product agroecosystems (Adler et al., 2012; Eliopoulos et al., 2002). Foraging *H. sylvanidis* females are attracted by odours emanating from the faeces of *T. confusum* larvae (Fürstenau et al., 2016). Lorenz et al. (2010) reported that females are able to penetrate 8 cm into the flour substrate to find *T. confusum*, but that the success in finding hosts declined with depth and also as particle size decreased. Hosts that are attacked are typically transported to a crevice or hole prior to oviposition (Ahmed et al., 1997; Lorenz et al., 2010). *Holepyris sylvanidis* females attack and feed on *T. confusum* larvae in the early instars and oviposit on larger larvae, normally laying a single egg onto each hosts, but 2 and 3 eggs clutches are occasionally observed (Ahmed et al., 1997). Notwithstanding these prior studies, obtaining empirical information on *H. sylvanidis* has been hampered by difficulties associated with collecting and then rearing this species under laboratory conditions. In this paper we report a viable laboratory-rearing protocol for *H. sylvanidis* along with observations on its behaviour and reproduction. Specifically, we describe and evaluate aspects of egg size, immature development, adult mating behaviour, pre-oviposition time, fecundity, host attack and use, and adult longevity. We discuss these new findings in regard to both the general biology of bethylid wasps and the potential for *H. sylvanidis* to suppress populations of pests.

2. Materials and methods

2.1 Host and parasitoid cultures

The host *T. confusum*, was reared in 12 cm diameter Petri dishes containing 20 g of *Triticum aestivum* flour and 5% of brewers' yeast as a substrate (Athanassiou and Kavallieratos, 2014). The *H. sylvanidis* used were a strain collected from wheat infested by *T. confusum* in a grain centre warehouse at Enna (Italy). Parasitoids were reared by keeping two female and one male *H. sylvanidis* in a 12 cm diameter Petri dish with fourth instar host larvae (Ahmed et al., 1997), with fresh fourth instar larvae added each week. Two squares (2 cm × 2 cm) of corrugated cardboard were placed into each Petri dish to provide places for female parasitoids to hide paralysed hosts (Ahmed and Islam, 1988). Adult *H. sylvanidis* host feed (Ahmed et al., 1997) but, in order to enhance egg production, were provided with a supplementary diet composed of water (1 ml), honey (0.1 g), pollen (7 grains) and royal jelly (0.05 g) absorbed onto a 1×1cm piece of filter paper. All cultures and experiments were maintained at 27±1°C and 65±5 % relative humidity.

2.2 Life history evaluations

We evaluated aspects of egg size, immature development, adult mating behaviour, pre-oviposition time, fecundity, host use and adult longevity.

2.3 Development of offspring

Parasitoid cultures were inspected daily and any fourth instar host larvae bearing a *H. sylvanidis* egg were collected kept individually in 4 cm diameter Petri dishes. The length and width of each egg was measured under a binocular dissecting microscope with image analysis software. The development of each *H. sylvanidis* offspring was observed daily until adult eclosion. Replicates in which the egg did not mature to adulthood were excluded. We recorded the number of days before the egg hatched (when head of the larva emerged from the chorion, according to Powel 1938), the durations of the larval and pupal stages and the sex of the emerging adult. We also recoded the time between each egg hatching and the penetration of the body of the host by the larva, the time taken for the host to be consumed and the time it took for larvae to spin a silken cocoon and pupate.

2.4 Mating behaviour

In each replicate, one male and one female *H. sylvanidis*, with no prior mating experience and within one day of eclosion as adults, were placed into a 4 cm diameter Petri dish and observed. In order to ensure that the adults were unmated, their cocoons had been isolated from the cultures for at least 4 days before adult emergence. Courtship and mating interactions were observed and the time until mating (pre-copulatory time) and the duration of copulation, from when the male mounted the female until the male dismounted, was recorded. After each replication, the Petri dish was cleaned with ethanol to remove any chemical stimuli.

2.5 Pre-oviposition period

In each replicate, a newly emerged (<1 day old) and unmated female was placed into a 12 cm diameter Petri dish containing a square of corrugated cardboard and 5 fourth instar *T. confusum* host larvae, which are the preferred host developmental stage (Ahmed et al., 1997). In some replicates a newly emerged male *H. sylvanidis* was also present, leading to the female being mated. The hosts were observed daily until the first *H. sylvanidis* egg was observed. Host larvae that were fed upon or had died and were no longer suitable for oviposition were replaced at each observation. The pre-oviposition period was calculated as the number of days between the emergence of the female and the first egg laid (Finlayson, 1950). There were 50 replicates using unmated and 50 using mated females.

2.6 Host attack and use

In each replicate (*n* = 10) one ≤5 day-old female was placed into a 12 cm diameter Petri dish containing 35 hosts, comprising 5 hosts of each of the 6 larval instars and 5 pupae. Host attack behaviour was observed. Each replicate was inspected daily for 10 days noting the number of hosts that had been fed upon or paralysed by the female. We note that it is difficult to detect the paralysis of host pupae but feeding damage was straightforward to observe.

2.7 Lifetime fecundity

In each replicate, a newly emerged female was placed into a 12 cm diameter Petri dish containing a square of corrugated cardboard and supplied with fourth instar host larvae twice per week: on Mondays, 5 fresh hosts were added to the Petri dish and on Fridays all larvae were removed and 10 fresh hosts provided. Hosts with eggs laid on them were collected on both Mondays and Fridays. The procedure was repeated until each female died. In some replicates a newly emerged male *H. sylvanidis* was also present from the start such that the female was mated.

2.8 Longevity

We evaluated the longevity of females (n = 92), with and without the provision of hosts and of males, with and without the provision of food (while adult males may not interact with hosts they could potentially feed on nectar and pollen). Mated females (<1 day old) were placed individually into plastic vials (2.6 cm × 0.6 cm) stoppered with cotton gauze. In some replicates, 5 fresh host larvae were provided twice per week and any previously provided hosts removed. In other replicates the females were starved. Male longevity was similarly evaluated (n = 144); males were either starved or provided with food on the gauze stopper twice per week. Some fed males received sucrose solution (0.5 g per 2.5 ml water) and others were provided with sucrose solution with 4 grains of pollen added. For both male and females, longevity was evaluated by inspecting the vial each day until the wasp died.

3. Statistical analysis

Where possible data were explored using generalized linear modelling (log-linear modelling, and parametric survival analysis; Crawley, 1993) but non-parametric Mann-Whitney U tests were employed when error distributions did not conform well to parametric assumptions. All tests were two-tailed and used the 0.05 significance level. Data were analysed using GenStat (version 17, VSN International, Hemel Hempsted) and StatPlus (version 6.0.3, AnalystSoft Inc.).

4. Results

4.1 Development of offspring

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

4.2 Mating behaviour

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

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

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

4.3 Pre-oviposition period

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

4.4 Host attack and use

When a female *H. sylvanidis* encountered a potential host she inspected it by touching its surface with her labial and maxillary palps. On recognizing a larva as a suitable host, the female immediately attacked it by grasping it with her legs and bending her abdomen around the larva and rotating her body by 360 degrees for three or four times. The attack is very rapid and it is difficult to observe exactly when the female stings the host with venom. The host was typically paralysed immediately and permanently (on occasion the host larva later regained activity and began to crawl, with a parasitoid egg adhering to its venter). Females continued to touch the host with their antennae for some seconds after stinging. While paralysed hosts were almost motionless they showed rhythmic movements of their legs. Sometimes the female attacked the legs of the host larva, biting the intersegmental membrane. Although each host larva was entirely explored, females paid most attention to the segments close to the legs. The females then typically grasped their host with their mandibles and moved it to another locality prior to oviposition, but some hosts were instead fed upon.

Of all hosts presented, 72.3% were utilized for feeding or were parasitized by *H. sylvanidis* females; of these, 39.1% were fed upon. Host acceptance and usage differed according to host developmental stage. Host acceptance increased as larvae had developed until the fifth instar and then declined (Fig. 4). In terms of usage, first instar larvae and pupae were only utilized for feeding, second and third instar larvae were commonly used for either feeding or oviposition while fourth, fifth and sixth instar hosts were almost exclusively use for oviposition (Fig. 3). As far as we could discern, each host was either fed upon or oviposited upon, but not both.

Ovipositing females usually laid a single egg onto the host, but clutches of two eggs were occasionally laid. Eggs were normally placed longitudinally between the first and second abdominal sternite, as figured in Reichmuth et al. (2007), but sometimes the egg was laid on the second and third sternite, or between the third and fourth abdominal sternites.

4.5 Lifetime fecundity

Females laid on average 16.42 (+SE = 0.871, -SE = 0.828) eggs during their lifetimes (*n* = 68). There was no significant difference in the overall fecundity realized by mated and unmated females (log-linear ANOVA corrected for overdispersion by assuming a quasi-

Poisson distribution of errors using an empirically estimated scale parameter: $F_{1,66} = 2.42$, $P = 0.124$, deviance explained = 3.5%). Egg laying activity was initially higher among mated females than unmated females and declined as females aged, with a more gradual decline among unmated females (Fig. 4a). The risk of a female dying increased between successive observation periods (fitting a Weibull model of cohort survival reduced the deviance significantly compared to an exponential model, $G_1 = 91.1$, $P < 0.001$) and unmated females lived significantly longer than mated females ($G_1 = 25.6$, $P < 0.001$, Fig. 4b). In many replicates females ceased laying eggs before all of the provided hosts had been oviposited on and several weeks before their time of death. The period of active reproduction was significantly shorter among mated females than among unmated females ($G_1 = 14.98$, $P<0.001$) but we observed that females continued to kill and eat hosts after ceasing to oviposit. The functions describing the models for mated and unmated females are respectively: = initial number × (Exp(-(0.0001219 × Observation period$^{3.684}$)) and = initial number × (Exp(-(0.00003438 × Observation period$^{3.684}$)).

4.6 Longevity

Female longevity was significantly greater when females were provided with fourth instar *T. confusum* larvae (48.6 ± 2.7 days) then when they were not (8.1 ± 0.5 days) (Weibull cohort survival model: $G_1 = 355.16$, $P < 0.001$): all unprovisioned females died within 20 days while females with hosts lived up to 86 days (Fig. 5a). Similarly, male longevity was affected by feeding treatment ($G_2 = 83.40$, $P < 0.001$, Fig. 5b): when starved males lived around 7 (± 0.2) days, males provided with sucrose lived on average for 18.18 (± 1.64) days and the males nourished with the sucrose solution and pollen lived on average for 35.48 (± 4.97) days. Although the survival of males fed with sucrose only and sucrose and pollen was initially similar (Fig. 5b), males fed with sucrose and pollen had significantly greater longevities ($G_2 = 21.10$, $P < 0.001$). The functions describing the models for the longevity of females and fed females are respectively: = 100 × (Exp(-(0,01574 × (Observation period^{1,928})))) and = 100 × (Exp(-(0,0005029 × (Observation period^{1,928})))). The functions describing the models for the longevity of males, males and sugar, males sugar and protein are the following: = 100 × (Exp(-(0,02237 × (Observation period^{1,928})))), = 100 × (Exp(-(0,0027559 × (Observation period^{1,928})))), =100 × (Exp(-(0,00058318 × (Observation period^{1,928})))).

5. Discussion

Holepyris sylvanidis is a natural enemy of immature beetles, acting either as a destructive host feeder or as a parasitoid of each suitable host encountered. As such its observed biology conforms broadly to expectations derived from knowledge of other species within the bethylid sub-family Epyrinae (e.g. Lauzière et al., 1999; Mayhew and Hardy, 1998; Pérez-Lachaud and Hardy, 1999). Further, host hiding prior to oviposition is exhibited in common with several other Epyrinae (Eliopoulos et al., in press; Griffiths and Godfray, 1988) as is laying single-egg, or otherwise, small clutches (Ahmed et al., 1997; Finlayson, 1950; Mayhew and Godfray, 1997; Mayhew and Hardy, 1998). The observed positions in which eggs were observed to be laid onto the hosts agree with patterns reported by Ahmed and Islam (1988).

Holepyris sylvanidis is protandrous, with males emerging in advance of females and mating occurs at the natal site. This observation agrees with Abdella et al. (1985). We observed that mating occurred soon after emergence and that males jump suddenly onto the female just prior to copulation, both observations tally with prior reports (Ahmed et al., 1997; Ahmed and Islam, 1988). Ahmed et al. (1997) further reported that females remain stationary during copulation, while we observed some females running around whilst *in copula*. The mating behaviour of *H. sylvanidis* is not complex and would not present obstacles to this species being mass reared for release as a biocontrol agent.

Holepyris sylvanidis is synovigenic (Ahmed et al., 1997) and host feeding likely serves to supply nutrients for egg maturation, as in other epyrine species (Lauzière et al., 1999; Pérez-Lachaud and Hardy, 1999). We observed that female *H. sylvanidis* fed primarily on host haemolymph but were also able to eat the tissues of the host larvae. Ahmed et al. (1997) have previously reported that *H. sylvanidis* females attack and feed on the first to the fourth larval instars but not the fifth and sixth, or the pupal stage. In contrast, we observed some feeding on all host developmental stages, although it was rare on fourth, fifth and sixth instar larvae which are primarily used for oviposition. The major discrepancy between our results and those of Ahmed et al. (1997) is that they reported an absence of host feeding on pupal *T. confusum* while we observed this commonly.

While host feeding leads to more hosts being killed by a current generation of adult parasitoids, it reduces the number of offspring produced per host found and as such may not be a desirable characteristic of beneficials as destructive host-feeders are predicted to suppress host population equilibria as less strongly than other parasitoids (Emerick and Singh, 2016; Jervis et al., 1996a, b; Jervis and Kidd, 1986). Host feeding could influence both the pre-oviposition period and the time required for laying successive eggs once a female has started to oviposit. For example, in the epyrine *Cephalonomia stephanoderis* Betrem the pre-oviposition period was shorter when there were more hosts available (Lauzière et al., 1999). As in several other epyrine species (Mayhew and Heitmans, 2000; Yamasaki, 1982) unmated female *H. sylvanidis* had longer pre-oviposition periods than mated females, possibly because there are fitness advantages to being able to produce both male and female offspring and thus virgin females may tend to delay the production of progeny to increase the probability of being mated before they oviposit (Godfray, 1990; Guertin et al., 1996).

We observed that fourth, fifth and sixth instar host larvae were usually used for oviposition, whereas Ahmed et al. (1997) found that only fourth instar larvae were oviposited on. It is likely that early instar hosts are too small to provide sufficient nutrients to support parasitoid development and are thus fed on (see above) but why no oviposition was observed on larger, fifth and sixth instar, hosts by Ahmed et al. (1997) is unclear. The fact that oviposition onto pupal *T. confusum* was observed by neither study suggests that biochemical changes during pupation make pupae nutritionally unsuitable for parasitoid development. Key nutritional differences between the developmental stages of *T. confusum* could potentially be identified using a metabolomics approach tailored to low biomass organisms (Kapranas et al., submitted; Snart et al., 2015) and the same approach could also be employed to understand better the range of host species it attacks (Eliopoulos et al., 2002).

The realized fecundity (a mean of 16.4 eggs laid by each female) of *H. sylvanidis* female is lower than estimates for other epyrines which are natural enemies of stored products pests: *Cephalonomia waterstoni* Gahan, 102 eggs (Finlayson, 1950) and *C. tarsalis*, 85 eggs (Powell, 1938) or 50-200 eggs (Lukáš, 2007). Unlike *C. tarsalis* (Cheng et al., 2003), mating status did not affect fecundity but abiotic environmental conditions could have influenced our estimate of fecundity, given that the epyrine *Prorops nasuta* Waterston is recorded as laying 322 eggs at 27°C and only 33 at 18°C (Infante, 2000). However, Abdella et al. (1985) found in a laboratory study optimum conditions for *H. sylvanidis* of 27°C and 57.7% relative humidity. This is close to the conditions in our study. We further found that *H. sylvanidis* females become egg limited as they age and thus do not oviposit on all suitable hosts provided. Some mated females ceased laying as much as 1-2 weeks before dying while in some unmated females oviposition ceased as many as 4 weeks before death.

The provision of hosts clearly affected female *H. sylvanidis* longevity, probably because provisioned females were able to host-feed to obtain nutrients for somatic maintenance, as well as egg production, extending their adult life expectancies from around 8 to around 48 days. Similarly, estimates of longevity of females of species in the epyrine genus *Cephalonomia* provided with hosts range between 30 and 94 days (Finlayson, 1950; Pérez-Lachaud and Hardy, 1999; Powell, 1938; van Emden, 1931; Yamasaki, 1982), depending on environmental conditions, while without hosts longevity is typically between 10-20 days (Yamasaki, 1982). We also found that unmated *H. sylvanidis* lived longer than mated females, in contrast to *C. tarsalis* in which both mated and unmated females lived an average of 35 days (Powell, 1938). Longevity can play an important role in the impact of parasitoids on pest populations (Hausmann et al., 2005).

Male parasitoids are typically shorter-lived than females; for instance, Powell (1938) reported that *C. tarsalis* males live for 6 days and our estimate of male *H. sylvanidis* longevity is 7 days. However, male *H. sylvanidis* longevity was greatly enhanced by the provision of nutrients. Male *H. sylvanidis* do not attack or feed on host larvae but may feed on non-host sources of sugar and proteins such as nectar and pollen. We found that providing sucrose alone approximately doubled male life-expectancy; similar enhancements have been reported in other parasitoid species (Fadamiro and Heimpel, 2001). Further providing both sucrose and pollen increased the average male *H. sylvanidis* longevity to 35 days, with some males living over 150 days, possibly because sugars could be used to aid the digestion of pollen (Sigsgaard et al., 2013). Further work will be required to establish how diet components influence longevity and thus how parasitoids should be fed most efficiently to enhance their life-histories (Hausmann et al., 2005). Fed males may also have enhanced courting, mating and dispersal abilities (Kaspi and Yuval, 2000) but the relative mating abilities of fed and unfed male *H. sylvanidis* have not yet been evaluated. Given that females must mate before being able to produce daughters and that only females attack populations of hosts (Ode and Hardy, 2008), enhancing the longevity of males and their abilities to find and mate with females may be beneficial in terms of improving the suppression of pest populations.

Although data from an industrial bakery and a mill suggest that *T. confusum* populations may be regulated by *H. sylvanidis* (Adler et al., 2012; Prozell and Schöller, 1998), there is limited evidence for the population dynamic interactions between *H. sylvanidis* and its hosts. The need to host-feed and a relatively low fecundity are unlikely to promote *H. sylvanidis* population growth and, if high parasitoid densities are reached, intra-specific mutual interference interactions may further limit its efficacy for biocontrol (Eliopoulos et al., in press).

6. Conclusions

Holepyris sylvanidis is a natural enemy of important stored product pests, most notably the flour beetle *Tribolium confusum,* and has the potential to be beneficially deployed for biological control, thus helping to decrease reliance on synthetic pesticides and maintain the organic status of some bakeries (Lorenz et al., 2010; Prozell and Schöller, 1998). While its ability to find hosts within the substrate is good (Lorenz et al., 2010), our data suggest that its realized fecundity is low and on that basis it seems unlikely that this species will have the capability of suppressing pest populations below an economic threshold after inoculative biocontrol release, as may be the case for other epyrine species that attack stored products pests (Eliopoulos et al., in press; Powell, 1938) or field crop pests (Batchelor et al., 2006). Given that many encountered hosts are killed by adult females feeding upon them, and that host-feeding enhances female longevity, *H. sylvanidis* may have better potential for inundative or augmentative deployment, largely as a predator, but whether this is economically feasible will depend on the efficiency of mass rearing methods. As shown here, the food supplemented during rearing can increase the number of progeny per female, but more research on mass-rearing is needed. Indeed, data from a flour mill suggest that *T. confusum* population suppression was achieved when several hundred *H. sylvanidis* were released every two weeks but ceased once parasitoid release stopped (Adler et al., 2012). However, the last mentioned study also proved the potential for establishment of *H. sylvanidis* after release. The longevity of this parasitoid makes it a good candidate for an inoculative release strategy implemented in an integrated control strategy, e.g. with effective sanitation programs and heat treatments (Schöller et al., 2006).

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Figure captions.

Table 1 Development time (days ± SE) of *Holepyris sylvanidis* eggs, larvae and pupae. Means within rows followed by different letters are significantly different (Mann-Whitney-U test, P<0.05).

Table 2. Components of *Holepyris sylvanidis* mating behaviour

Fig. 1. Schematic representation of the course of events in a typical sequence of mating behaviour. Each of the main behaviours are reported in upper case, with successive component behaviours reported in lower case.

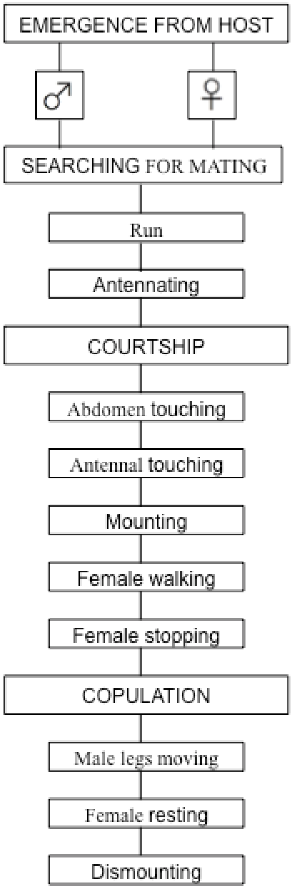
Fig. 2. Pre-oviposition period for unmated and mated *H. sylvanidis* females. * = mean significantly different, Mann-Whitney U-test, $P < 0.05$

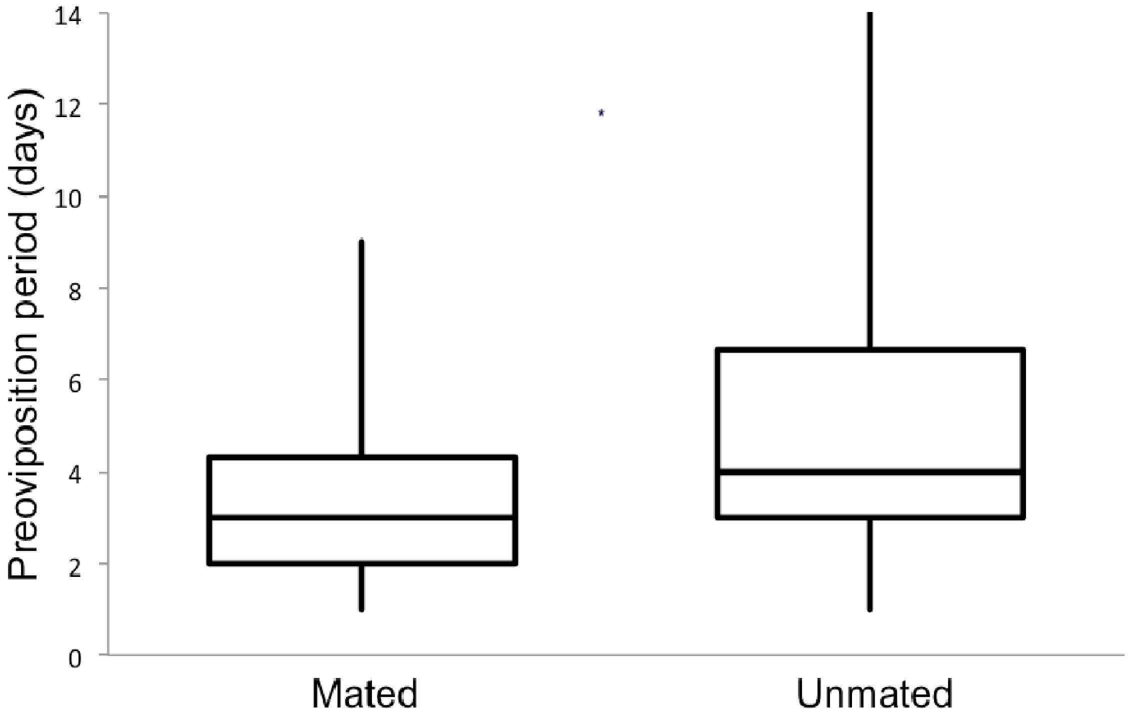
Fig. 3. The use of presented hosts over ten across ten replicates, with 5 hosts of each of the 7 stages present per replicate. The maximum number of hosts of a given stage that could be attacked was 50. Bars are shown stacked.

Fig. 4. Egg production and longevity of females supplied with hosts twice per week until death. There were two observation periods per week, such that 30 periods represents ≈105 days. Panel (a) shows the egg production by females alive at a given period. Panel (b) shows the survival of the same cohorts of females: Data are shown as dashed lines and the models fitted using Weibull survival analysis are shown as smooth curves.

Fig. 5. Cohort survival of females (a) and of males (b). Data are shown as dashed lines and the models fitted using Weibull survival analysis are shown as smooth curves.

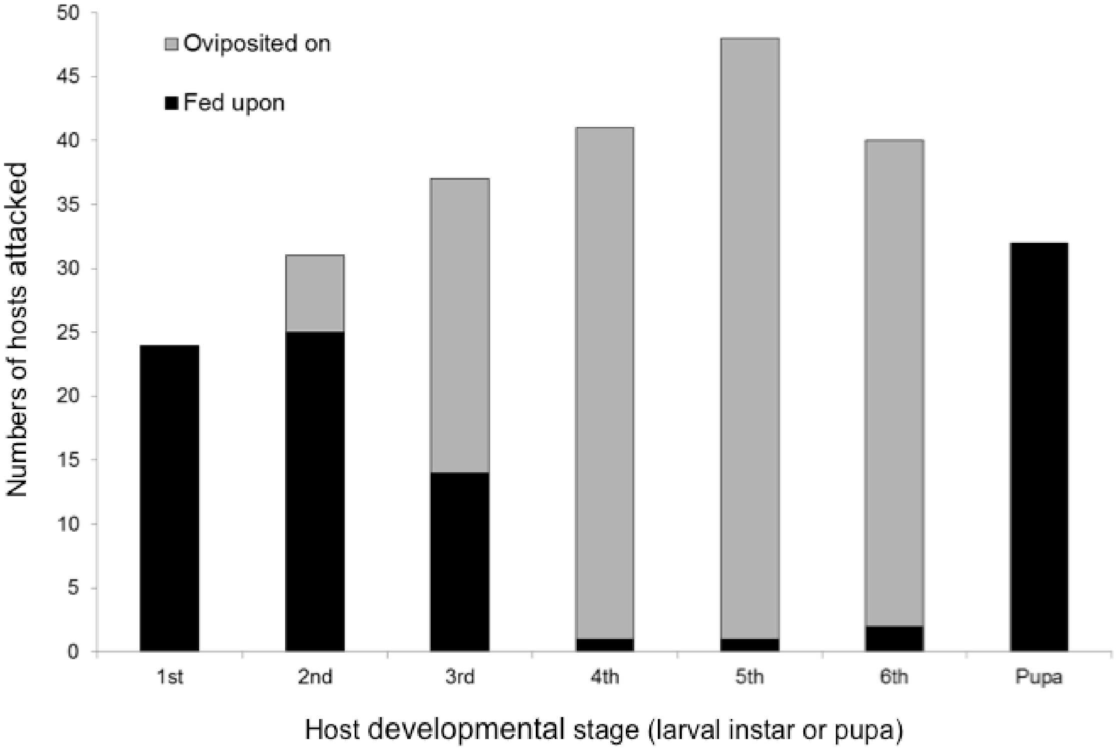
Graphical abstract Development of the parasitoid *H. sylvanidis*: the female laid and egg longitudinally between the first and second abdominal sternite of the host larvae (a), the parasitoid larvae developed as semi-ectoparasitoid (b), and spun a cocoon some centimetres away after the host body was devoured (c,d). The overall development time changed significantly between the two sexes of *H. sylvanidis*.


EMERGENCE FROM HOST
♂
♀
SEARCHING FOR MATING
Run
Antennating
COURTSHIP
Abdomen touching
Antennal touching
Mounting
Female walking
Female stopping
COPULATION
Male legs moving
Female resting
Dismounting



645

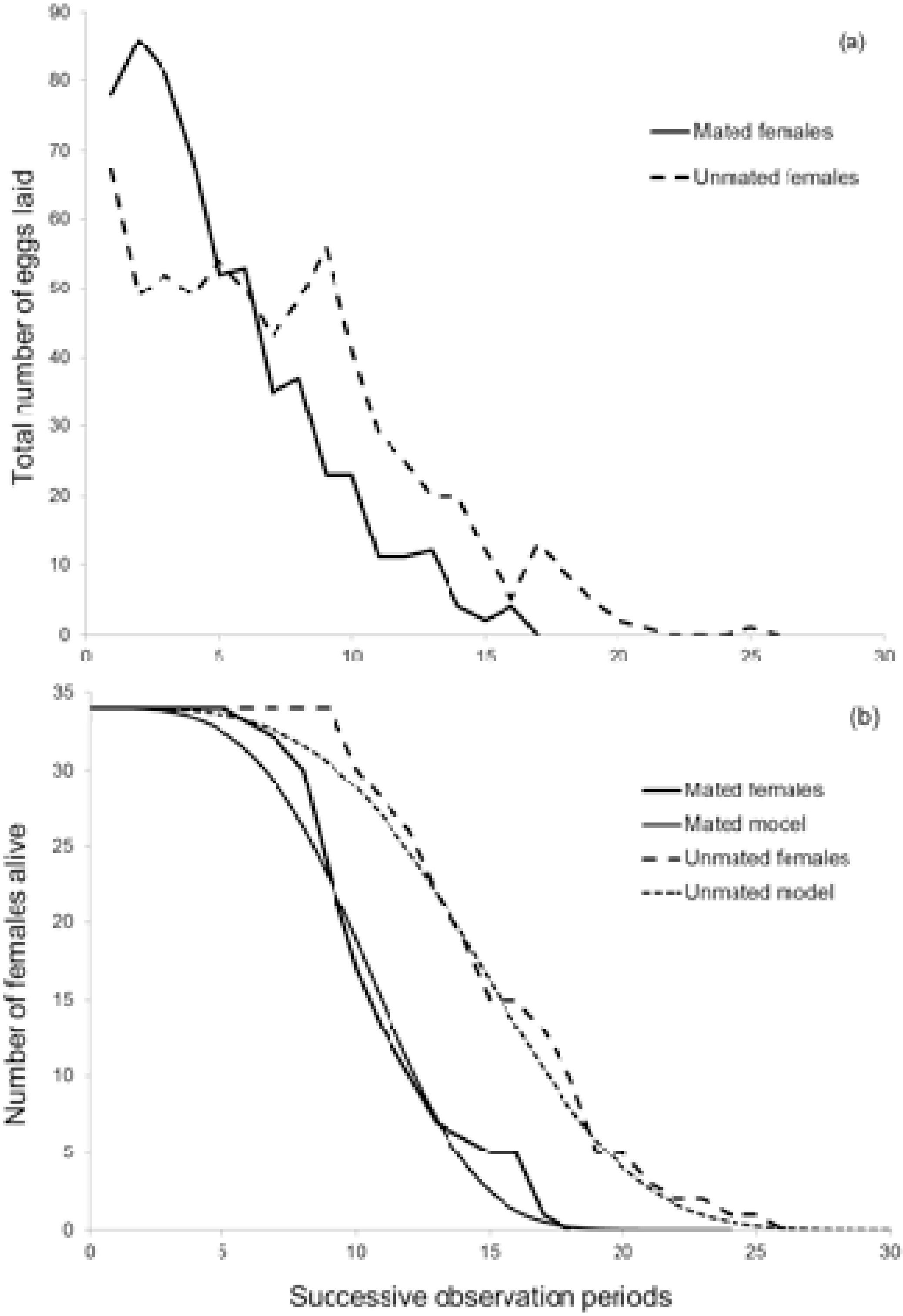
646

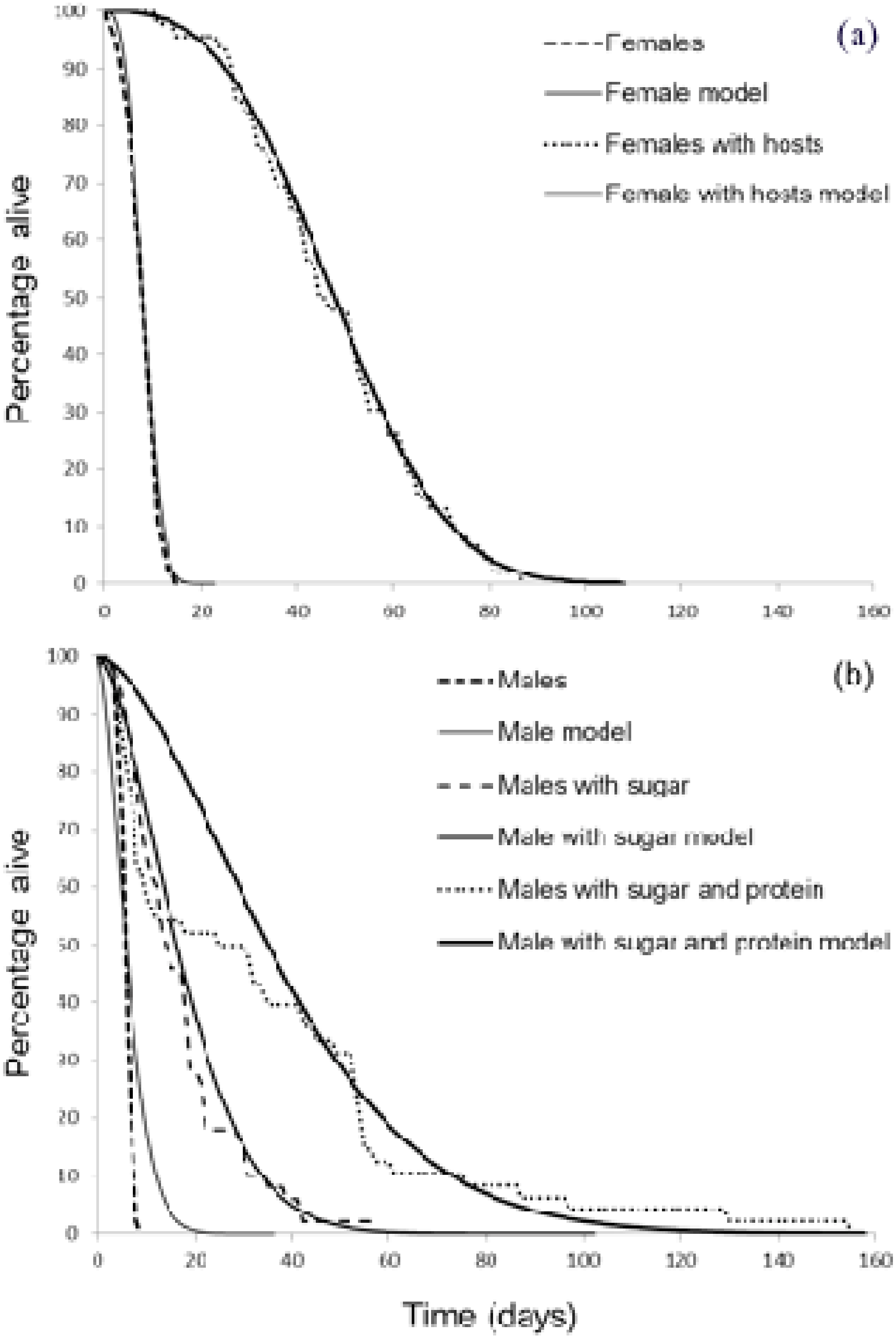


50
45
40
35
30
25
20
15
10
5
0
Numbers of hosts attacked
▨ Oviposited on
■ Fed upon
1st
2nd
3rd
4th
5th
6th
Pupa
Host developmental stage (larval instar or pupa)

647

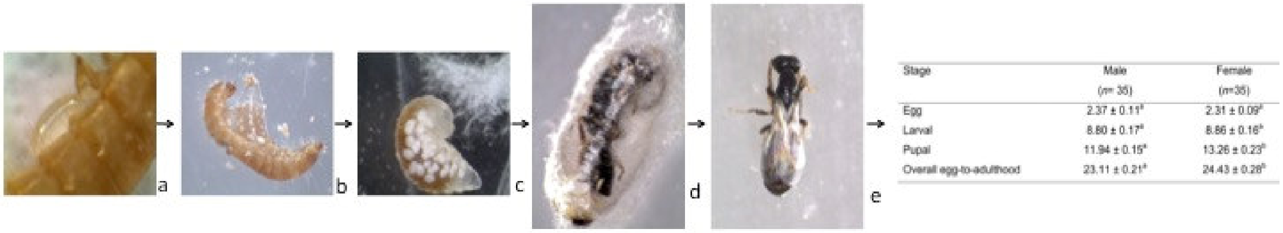
648


(a)
Total number of eggs laid
Mated females
Unmated females
0
10
20
30
40
50
60
70
80
90
0
5
10
15
20
25
30
(b)
Number of females alive
Mated females
Mated model
Unmated females
Unmated model
0
5
10
15
20
25
30
35
Successive observation periods


(a)
----Females
Female model
Females with hosts
Female with hosts model
Percentage alive
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100
120
140
160
(b)
Males
Male model
Males with sugar
Male with sugar model
Males with sugar and protein
Male with sugar and protein model
Percentage alive
Time (days)

651

652



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^{a}
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}

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^{a}
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}

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

- The mating status significantly influences the pre-oviposition period of *H. sylvanidis*
- The mating status does not influence the fecundity of *H. sylvanidis*
- A food source significantly influenced the longevity of female and male
- The life cycle lasts for 23 days in male and 24 days in female at 27 °C