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Research article

Influences of Temperature and Ootheca Age on the Life History of the Cockroach Ootheca Parasitoid *Aprostocetus hagenowii* (Hymenoptera: Eulophidae)

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Abstract

The influences of ootheca age and temperature on the life history of *Aprostocetus hagenowii* (Ratzeburg) (Hymenoptera: Eulophidae), a gregarious ootheca parasitoid of the American cockroach *Periplaneta americana* (L.) (Dictyoptera: Blattidae), were evaluated. Oothecae were incubated at 20, 25, and 30 °C to produce oothecae aged 1–60, 1–40, and 1–30 d old, respectively. Fitness traits (development time, percentage emergence, number of progeny, percentage female progeny, and female body size) of *A. hagenowii* developing in these different-aged oothecae were determined. For oothecae incubated at 20, 25, and 30 °C, parasitoids successfully developed in oothecae aged up to 50, 30, and 20 d old, which represent 72.9%, 65.9%, and 61.9% of the total embryonic development time of *P. americana*, respectively, without any changes in their fitness traits. When *A. hagenowii* from oothecae kept at constant temperatures (20, 25, 30, 32, and 35 °C) were compared, the immature development time (71.0–34.0 d) and adult life span decreased with increasing temperature. No parasitoid emerged at 35 °C. The lower, upper, and optimal temperature-dependent developmental thresholds were 9.5, 34.2, and 31.1 °C, respectively. Thermal constant for total immature development was 666.7 degree-days. Temperature did not affect lifetime realized fecundity and number of oothecae parasitized by females but did influence parasitism activities over time. Sugar-fed females sustained longer periods of high parasitism rates (≥70%) at 20–30 °C (15–30 d) than at 32–35 °C (1–5 d). These results are useful for determining the ootheca age and temperature range optimal for parasitoid rearing and for estimating the effectiveness of biological control by the wasps.

Key words: ootheca parasitoid, egg age, temperature threshold, *Periplaneta americana*

The American cockroach, *Periplaneta americana* (L.) (Dictyoptera: Blattidae), is a major insect pest of economic and public health importance (Rust 2008). It is an indoor and outdoor species found in tropical and subtropical regions around the world (Cornwell 1968). In addition to finding shelter in outdoor microhabitats (e.g., tree holes, plumbing chases, and crevices around buildings), American cockroaches breed in large numbers in waste disposal structures (e.g., drainage systems, sewers, and bin chutes), where warm and humid conditions and waste organic matter promote their growth (Pawson and Gold 1993, Suiter et al. 1998, Lee and Ng 2009, Tee et al. 2011a). These outdoor populations tend to invade buildings and establish indoor infestations (Eads et al. 1954, Robinson 2002), where they potentially can be vectors of disease by passing pathogens from their habitats to food and food preparation areas (Roth and Willis 1957, Reuger and Olson 1969, Lee 1997). Thus, infestations pose a potential health threat in human living environments.

The eggs of *P. americana* are enclosed in a hardened bean-shaped case called an ootheca (ootheca generally refers to the case and the cockroach eggs contained inside). Oothecae often are deposited in hidden locations and covered with debris by females (Rau 1943, Piper et al. 1978, Yeh 1995). Therefore, application of liquid insecticides may not reach them, rendering re-infestation an issue in managing this cockroach.

Surveys of natural blattid cockroach populations revealed that oothecae are commonly parasitized by the ootheca parasitoid *Aprostocetus hagenowii* (Ratzeburg) (Hymenoptera: Eulophidae). Parasitism rates ranged from 13 to 84% (Cameron 1955, Fleet and Frankie 1975, Kanayama et al. 1976, Piper et al. 1978, Narasimham and Sankaran 1979). This eulophid parasitoid is a gregarious species, with up to 96 wasps being produced a parasitoid (Tee et al. 2010). Thus, biological control of *P. americana* with *A. hagenowii* releases may be an effective way to manage cockroach

populations. Several studies have investigated how ootheca age affects parasitoid production and how treatment or cold storage of oothecae affects parasitoid rearing (Suiter et al. 1998, Tee and Lee 2013). Vinson (2010) reported that resources within a host egg convert from simple nutritional contents to more complex compounds with age, and beyond a certain point of embryogenesis an egg becomes unsuitable for parasitoid development. Because incubation time of *P. americana* oothecae varies between 40 and 69 d at 20–30 °C (Bressan-Nascimento et al. 2008) and because incubation time was not reported in previous ootheca age studies, it remains unclear what percentage of *P. americana* embryonic development is beyond the stage suitable for parasitoid rearing. Host age also critically influences various fitness traits of parasitoid wasps, including development time, successful parasitism, and body size, and it affects females' oviposition decisions (King 1990, Ueno 1997, Sousa and Spence 2001, Wei et al. 2014). Thus, studies of the effect of host age on immature *A. hagenowii* development and on the oviposition preference of females are important to improve parasitoid rearing and to predict parasitism of wild oothecae in biological control release program.

Parasitoid development occurs between a lower and upper temperature threshold (beyond which development ceases) and at maximum rate at optimum temperature. Besides growth rate, rearing temperature also affects the fitness traits of ensuing adult wasps, including size, longevity, fecundity, and nutrient reserves (Chen et al. 2006, Colinet et al. 2007), and likely have consequences for their use as biological control agents. For adult parasitoids, most species carry limited teneral reserves accumulated during larval development (Visser and Ellers 2008, Visser et al. 2010). These reserves are used for reproduction, survival, and locomotion (Jervis et al. 2008), and their depletion is likely temperature dependent. Thus, environmental temperature may play a role in influencing the survivorship and parasitism capacity of adult wasps. This is relevant to biological control because releases of *A. hagenowii* in urban settings are directed at primary harborage sites of *P. americana* around and in buildings (such as plumbing chases, treeholes, sewers, and crevices), which experience variation in microclimate. Hence, it is critical to investigate how temperature affects the development and performance of *A. hagenowii* in order to optimize their rearing and use as a biological control agent of *P. americana*.

In this study, we first examined the influence of the age of oothecae incubated at 20, 25, and 30 °C on several biological parameters (% emergence, developmental time, sex ratio, and hind tibia length [HTL, as a proxy of body size]) of *A. hagenowii*. The effect of constant temperature (20, 25, 30, 32, and 35 °C) on developmental parameters (% emergence, developmental time, and sex ratio) and temperature thresholds (optimum, lower, and upper values) of the parasitoid then were studied. We also assessed the influence of temperature on the survival, parasitism activities, and lifetime realized fecundity of *A. hagenowii*.

Materials and Methods

Insect Rearing

Periplaneta americana and *A. hagenowii* were collected from Penang, Malaysia, and maintained in the Urban Entomology Laboratory, Universiti Sains Malaysia (USM), using the methods described by Tee and Lee (2013). Newly laid *P. americana* oothecae used in the experiments were collected from pieces of Styrofoam (15 by 10 by 3 cm), which had been placed inside *P. americana* rearing containers (45 by 30 by30 cm, 100–120 adult females and 10 adult males) from 1700 to 0800 daily. To provide *A. hagenowii* wasps for the experiments, ≤1-wk-old oothecae were individually exposed to parasitism by an *A. hagenowii* female inside a 2-ml microtube with a perforated lid and held at 26.4 ± 0.2 °C and 63.3 ± 0.2% relative humidity (RH). Upon parasitoid emergence, wasps were 1) transferred for use in the survival and parasitism activity experiments and 2) allowed to sib-mate for 6 h inside the tube before being used as 1-d-old oviposition-inexperienced females in experiments examining ootheca age effect on parasitoid fitness and the influence of temperature on lifetime realized fecundity.

Influence of Age of Oothecae Incubated at Different Temperatures on Fitness Traits of *A. hagenowii*

In this experiment, the influence of ootheca age on fitness traits of *A. hagenowii* development was examined using oothecae incubated at three different temperatures (20, 25, and 30 °C).The age classes tested for oothecae incubated at 1) 20 °C were 1-, 10-, 20-, 30-, 40-, 50-, and 60 d old; 2) 25 °C were 1-, 10-, 20-, 30-, and 40 d old; and 3) 30 °C were 1-, 10-, 20-, and 30 d old. These temperatures and ootheca ages were selected based on a previous study of *P. americana* embryonic development, which reported oothecae were most viable at 20, 25, and 30 °C, with incubation time of 68.8, 48.3, and 39.6 d, respectively (Bressan-Nascimento et al. 2008). For each incubation temperature, five replicates (100-ml plastic containers) of five oothecae ($n = 5$) were assigned for each ootheca age and a control set (without exposure to parasitoids). One-day-old oothecae were placed inside environmental chambers and held at 20, 25, and 30°C and a RH of 55–65% at 0900. Oothecae without exposure to parasitoids (control set) were checked daily to determine the embryonic development time of *P. americana* at each incubation temperature. At each time interval, oothecae were retrieved from each chamber and exposed individually to a 1-d-old *A. hagenowii* female inside a 2-ml microtube with a perforated lid. *Aprostocetus hagenowii* females were removed after 24 h. These exposed oothecae were maintained under environmental conditions similar to those used for parasitoid rearing and checked daily for parasitoid emergence for up to 60 d post parasitism exposure. Upon parasitoid emergence, the number and sex of parasitoids was determined. Additionally, one female was randomly selected from each clutch and observed under an SZ61 stereomicroscope (Olympus, Tokyo, Japan) equipped with IC Imaging Standard, version 2.1 (The Imaging Source Europe GmbH, Bremen, Germany). The right HTL was measured, as an indicator of body size, using Analysis Image Processing software (Soft Imaging System GmbH, Münster, Germany). From this experiment, we determined percentage emergence (number of oothecae with parasitoid emergence divided by the number of exposed oothecae × 100), number of progeny, percentage female progeny, and HTL.

Influence of Temperature on Development of Immature *A. hagenowii*

Aprostocetus hagenowii females were transferred individually into a 2-ml microtube with a perforated lid containing a ≤ 1-d-old ootheca. Wasps were observed to determine whether they had parasitized the ootheca. Oothecae were considered to have been parasitized if *A. hagenowii* females were observed to complete this sequence of oviposition behaviors (as outlined by Roth and Willis, 1954). Thirty *A. hagenowii*-parasitized oothecae were selected and randomly allocated to develop under five constant temperatures (20, 25, 30, 32, and 35°C, each with 30 oothecae) at an RH of 55–65%. Parasitoid emergence was checked daily for 120 d. The number and sex of parasitoids per clutch were determined.

Influence of Temperature on Survival, Parasitism Activities, and Lifetime Fecundity of *A. hagenowii*

Survival, parasitism activities, and lifetime fecundity were evaluated under two food conditions (water only and 10% sugar solution) at 20, 25, 30, 32, and 35 °C. The 35 °C ultimately was excluded from fecundity because no parasitoid development occurred at this temperature (see results). In the survival experiment, one clutch of newly emerged *A. hagenowii* was transferred into a 100-ml polyethylene container with a screened lid. Water or a 10% sugar solution was provided from a cotton-plugged 2-ml microtube. This set up was replicated six times for each combination of temperature and food condition. Containers were held in environmental chambers at 20, 25, 30, 32, or 35 °C and an RH of 55–65%. Survival of parasitoids was checked and dead parasitoids were removed daily.

For the parasitism experiment, four clutches of newly emerged *A. hagenowii* were transferred to one of the five 1,000-ml polyethylene containers covered with a screen lid. Two-thirds of the inner wall of the container was coated with a thin layer of fluon (Asahi Glass Company, Tokyo, Japan) to prevent the wasps from crawling up the wall. Water or a 10% sucrose solution was provided from a 2-ml microtube plugged with cotton. This set up was replicated five times (containers) for each temperature. Containers were held at 20, 25, 30, 32, or 35 °C and an RH of 55–65%. After 1 d, four *A. hagenowii* females were randomly selected from each container and each parasitoid was provided a ≤ 2-d-old ootheca to parasitize inside a 2-ml microtube. Wasps and oothecae were held at their target temperature for 24 h. After 24 h, females were removed and oothecae were maintained under environmental conditions similar to those used for parasitoid rearing. This procedure was repeated daily for parasitoids provided only water and at 5-d intervals for parasitoids provided the 10% sucrose solution. The experiment was terminated when one of the five containers had less than four female survivors. Daily for 60 d oothecae were check daily for parasitoid emergence. After 60 d, oothecae without parasitoid emergence were dissected and determined to be parasitized if dead immature parasitoids were detected. Parasitism rate of ootheca was defined as the number of oothecae parasitized divided up by the number of exposed oothecae × 100.

For the lifetime fecundity experiment, one 1-d-old mated and oviposition-inexperienced female was transferred into a 100-ml polyethylene container. Water or a 10% sugar solution was supplied inside the container in cotton-plugged 2-ml microtube. Ten newly laid oothecae were placed into each container on which the female could oviposit. These containers were then placed into environmental chambers set at constant temperatures of 20, 25, 30, and 32 °C. Survival of parasitoids was checked daily. For females that survived more than 20 d and 40 d (e.g., at 20 and 25 °C), the 10 oothecae were removed and replaced with another 10 newly laid oothecae at 20-d intervals. This provided a sufficient number of oothecae, as Roth and Willis (1954) reported that the average number of oothecae a female *A. hagenowii* can parasitize in its entire lifetime was 2.1 oothecae when several oothecae were presented simultaneously and 4.1 oothecae when oothecae were provided daily. The 20-d exposure period of oothecae was selected because oothecae incubated at 32 °C became unsuitable for parasitoid development as they reached >20 d old (see Fig. 1B). To measure parasitoid emergence, the exposed oothecae were transferred individually into a 2-ml microtube with a perforated lid and maintained under environmental conditions similar to those used for parasitoid rearing. This experiment was replicated 12 times for each combination of temperature and food condition. The numbers of progeny produced and oothecae parasitized were determined for each female.

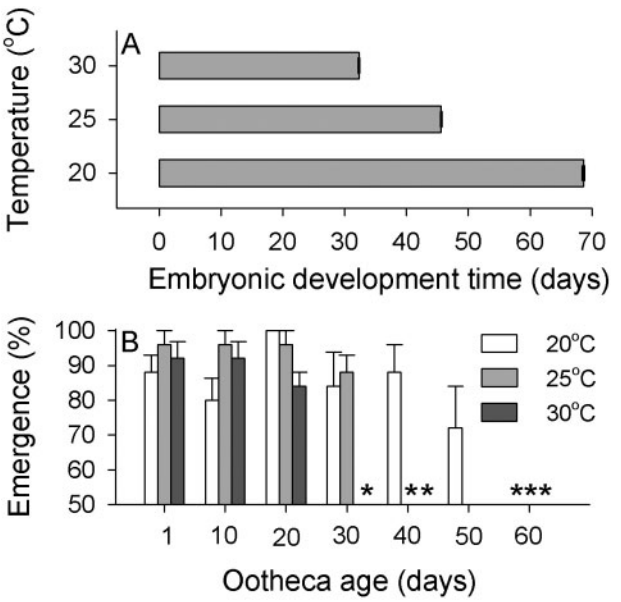

Fig. 1. (**A**) *Periplaneta americana* embryonic development time at 20, 25, and 30 °C ($n = 5$). (**B**) Percentage emergence of *A. hagenowii* from different-aged oothecae incubated at 20, 25, and 30 °C. Asterisk *, **, and *** indicate no wasps emerging from oothecae aged 30 d at 30 °C, 40 d at 25 °C, and 60 d at 20 °C, respectively. Vertical line on each bar indicates standard error of the mean ($n = 5$).

Data Analysis

We used linear and nonlinear models to determine the temperature-dependent developmental thresholds of *A. hagenowii*. For the linear model, the relationship between developmental rate (Dr, developmental time in d^{-1}) and temperature (T) was described using the linear equation $Dr = aT + b$, where a and b were constants estimated using least square regression analysis. The lower temperature developmental threshold (T_{lower}) and thermal constant (K) were determined as $T_{lower} = -b/a$ and $K = 1/a$ degree-days (DD), respectively, by extrapolating the regression line of the linear portion of the relationship (Campbell et al. 1974). Thus, only data from 20 to 30 °C were used in the linear regression analysis. For the nonlinear model, Dr was regressed against T using the model developed by Logan et al. (1976) and modified by Lactin et al. (1995) as follows:

$$Dr(T) = e^{\rho T} - e^{(\rho T_{max} - (T_{max} - T)/\Delta)} + \lambda$$

where Dr (T) is developmental rate at temperature T (°C), ρ is rate of increase from the lower temperature threshold to optimum temperature, T_{max} is lethal temperature at which biological processes can no longer be maintained, Δ is the temperature range between T_{max} and the temperature at which thermal breakdown overrides development, and λ is a parameter that renders the curve to intercept the x-axis, thus allowing the lower temperature developmental threshold to be estimated. The parameters ρ, T_{max}, Δ, and λ were estimated with nonlinear regression analysis using the Marquardt algorithm method. The optimum temperature (T_{opt}) was calculated using the following equation described by Logan et al. (1976): $T_{opt} = T_{max}[1 + \varepsilon \ln(\varepsilon b_o)/(1 - \varepsilon b_o)]$, where $\varepsilon = \Delta/T_{max}$ and $b_o = \rho T_{max}$. Lower and upper temperature developmental thresholds (T_{lower} and T_{upper}, respectively) were determined graphically using SigmaPlot version 10 (Systat Software Inc, San Jose, CA).

The percentage emergence, developmental time, number of progeny, percentage female progeny, and HTL were analyzed using one-way analysis of variance (ANOVA). Percentage data were arcsine

square-root-transformed before being subjected to ANOVA (Conover and Iman 1981). When a significant difference was detected, we used Tukey's HSD post hoc test to separate means between treatment groups (ootheca age classes and constant temperatures). Survival data were analyzed using Kaplan–Meier survival analysis and compared for differences between temperatures using log-rank tests. Two-way ANOVA with temperature (20, 25, 30, and 32 °C) and food condition (water alone and 10% sugar solution) as the main effects was used to analyze the effect of these factors on the lifetime realized fecundity and number of oothecae parasitized by females. When a significant main effect of food condition was detected, we used Student's t-test to compare difference between food conditions at each temperature. Parasitism activity (%) at each temperature was analyzed using the nonparametric one-way Kruskal–Wallis test, and difference between days was compared using Dunn's multiple comparison of rank test. Nonlinear regression analysis, the Kruskal–Wallis test, and Dunn's multiple comparison of rank test were conducted using JMP Pro version 10 (SAS Institute Inc, Cary, NC), whereas all the other statistical analyses were carried out using SPSS version 20 (IBM Corp, New York, NY). The level of significance $\alpha = 0.05$ was set for all statistical analyses.

Results

Influence of Age of Oothecae Incubated at Different Temperatures on Fitness Traits of *A. hagenowii*

Embryonic development times of *P. americana* were 68.6, 45.6, and 32.3 d at 20, 25, and 30 °C, respectively (Fig. 1A). For oothecae incubated at these temperatures, parasitoids successfully developed in oothecae aged up to 50 d old (72.9% of total embryonic development time [EDT]), 30 d old (65.9% EDT), and 20 d old (61.9% EDT), respectively (Fig. 1B). No parasitoid wasps emerged from 60-, 40-, and 30-d-old oothecae incubated at 20, 25, and 30 °C, respectively. Percentages of parasitoid emergence from different-aged oothecae incubated at 20, 25, and 30 °C were 72–88%, 88–96%, and 84–92%, respectively. There was no significant difference in percentage parasitoid emergence among different-aged oothecae at each incubation temperature (20 °C: $F_{5,24} = 1.401$, $P = 0.259$; 25 °C: $F_{3,16} = 0.889$, $P = 0.468$; 30 °C: $F_{2,12} = 1.000$, $P = 0.397$; Fig. 1B). Ootheca age did not significantly affect development time (Fig. 2A; 20 °C: $F_{5,120} = 0.744$, $P = 0.592$; 25 °C: $F_{3,90} = 0.228$, $P = 0.877$; 30 °C: $F_{2,64} = 2.267$, $P = 0.112$), percentage female progeny (Fig. 2C; 20 °C: $F_{5,120} = 1.916$, $P = 0.097$; 25 °C: $F_{3,90} = 2.603$, $P = 0.057$; 30 °C: $F_{2,64} = 1.18$, $P = 0.314$), or HTL (Fig. 2D; 20 °C: $F_{5,120} = 1.483$, $P = 0.200$; 25 °C: $F_{3,90} = 1.122$, $P = 0.345$; 30 °C: $F_{2,64} = 0.358$, $P = 0.701$) of *A. hagenowii* at any incubation temperature. Overall, parasitoids development time was 38.5–40.6 d, mean percentage female progeny ranged from 91.5 to 94.5%, and mean female HTL ranged from 0.50 to 0.52 mm.

The number of wasps produced from oothecae incubated at 20 and 25 °C were significantly influenced by ootheca age (20 °C: $F_{5,120} = 3.495$, $P = 0.006$; 25 °C: $F_{3,90} = 15.623$, $P < 0.001$; 30 °C: $F_{2,64} = 3.023$, $P = 0.056$; Fig. 2B). At incubation temperatures of 20 and 25 °C, 50- and 30-d-old oothecae produced a mean of 69.7 and 73.3 wasps, which were significantly lower than the 84.5 and 80.5–85.5 wasps produced from 1- and 1–20-d-old oothecae, respectively.

Influence of Temperature on Development of *A. hagenowii*

No parasitoids emerged from oothecae held at 35 °C (Table 1). Emergence rates of parasitoids at 20–32 °C ranged from 86.7 to 90.0%, and no significant difference was found among these percentages ($F_{3,20} = 1.68$, $P = 0.206$). Parasitoids took significantly longer to develop at 20 °C and shorter to develop at 30–32 °C ($F_{3,107} = 1814.5$, $P < 0.001$). Parasitized oothecae held at 20 °C produced significantly fewer wasps than those held at 25 and 32 °C ($F_{3,107} = 6.67$, $P < 0.001$). Rearing temperature did not significantly influence sex ratio ($F_{3,107} = 2.12$, $P = 0.101$). The relationship between temperature and developmental rate of *A. hagenowii* was adequately described by linear and nonlinear models (Fig. 3). The linear model estimated the values of T_{lower} and K for immature development to be 9.53 °C and 666.7 DD, respectively. The T_{lower}, T_{upper}, and T_{opt} estimated using the nonlinear model were 10.3, 34.2, and 31.1 °C, respectively.

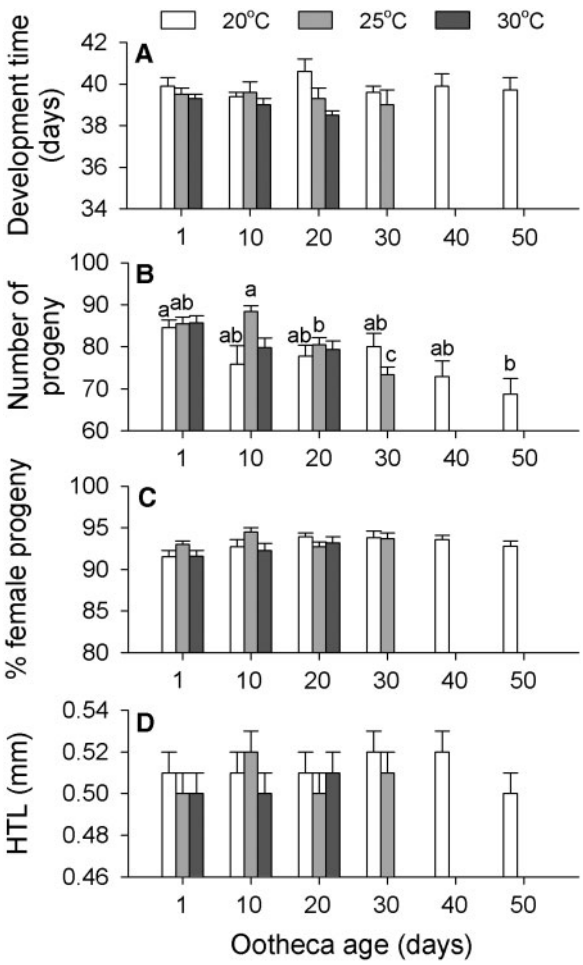

Fig. 2. Development time (**A**), number of progeny produced/ootheca (**B**), percentage female progeny/ootheca (**C**), and HTL (**D**) of *A. hagenowii* from different-aged oothecae incubated at 20, 25, or 30 °C. For each incubation temperature, bars with different letters indicate a significant difference among different-aged oothecae (Tukey's HSD test, $P < 0.05$). Number of replicates for each ootheca age at each incubation temperature: 20 °C: 1, 10, 20, 30, 40, and 50 d = 22, 20, 25, 21, 22, and 18; 25 °C: 1, 10, 20, and 30 d = 24, 24, 24, and 22; 30 °C: 1, 10, and 20 d = 23, 23, and 21, respectively. Vertical line on each bar indicates standard error of the mean.

Influence of Temperature on Survival and Parasitism Activities of *A. hagenowii*

Male and female parasitoids lived a significantly shorter time as temperature increased from 20 to 35 °C when fed either water alone (log-rank test: males, $\chi^2_4 = 109.4$, $P < 0.001$; females, $\chi^2_4 =$

Table 1. Mean (± SE) percentage emergence, development time (d), number of progeny/ootheca, and percentage female progeny of *A. hagenowii* emerged from *P. americana* oothecae at 20–35 °C

Temp. (°C)	*n*	Emergence (%)	*n*	Development time, days (range)	No. progeny/ootheca	% Female progeny
20	6	90.0 ± 4.5a	27	71.0 ± 0.6 (68–82)a	78.6 ± 1.8a	90.0 ± 0.7a
25	6	96.7 ± 3.3a	29	42.8 ± 0.4 (40–48)b	88.6 ± 1.3b	90.2 ± 0.7a
30	6	96.7 ± 3.3a	29	34.7 ± 0.2 (33–37)c	83.1 ± 1.4ab	90.0 ± 0.6a
32	6	86.7 ± 4.2a	26	34.0 ± 0.3 (31–38)c	85.6 ± 2.0b	92.0 ± 0.4a
35	6	0		–	–	–

Means in the same column followed by different letters are significantly different (Tukey's HSD, $P < 0.05$, n = number of replicates).

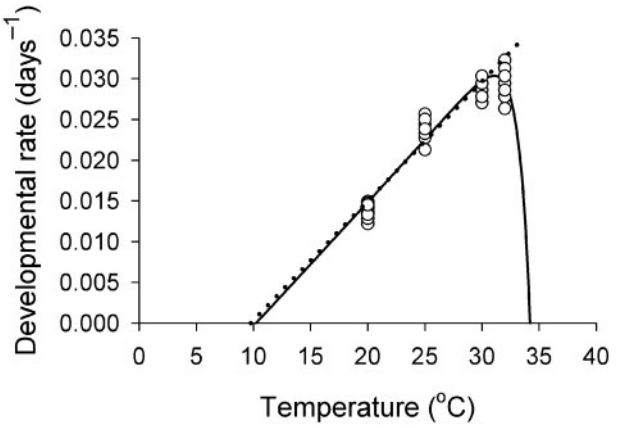

Fig. 3. Linear (dotted line) and nonlinear model (solid line) fitted to the observed developmental rates (days^{-1}) (white circles) of *A. hagenowii* at 20–32 °C. Number of replicates for 20, 25, 30, and 32 °C = 27, 29, 29, and 26, respectively.

2360.7, $P < 0.001$) or a 10% sucrose solution (males, $\chi^2_4 = 111.1$, $P < 0.001$; females, $\chi^2_4 = 2875.6$, $P < 0.001$; Fig. 4). Males and females fed water alone lived 2.0–4.4 d and 3.6–9.2 d at 20–35 °C, respectively. When a 10% sucrose solution was offered, mean survival times of males was 18.3, 15.8, 12.5, 11.6, and 6.6 d and 32.0, 23.6, 20.2, 14.5, and 6.6 d for females at 20, 25, 30, 32, and 35 °C, respectively. The number of oothecae parasitized and lifetime fecundity of *A. hagenowii* were significantly affected by food condition ($F = 71.75$, df = 1, $P < 0.001$) but not by temperature ($F = 0.787$, df = 3, $P = 0.505$) or the temperature × food condition interaction ($F = 1.01$, df = 3, $P = 0.391$). At 20–32 °C, females reared with 10% sugar solution successfully parasitized 3.3–4.1 oothecae (Fig. 5A) and produced 131.9–153.8 progeny (Fig. 5B), and these values were significantly greater than the 2.0–2.2 oothecae parasitized and 89.7–98.5 progeny produced by females provided water only (at each temperature: $P < 0.05$, Student's *t*-test; Fig. 5).

When water alone was provided, parasitism rates of oothecae by *A. hagenowii* females at 20–25, 30, and 32 °C ranged from 80 to 95% at d 1–3, 1–2, and 1, respectively (Fig. 6). These high parasitism rates decreased thereafter and were significantly lower at d 6, 5, and 3, respectively (20 °C: $H_6 = 27.14$, $P < 0.001$; 25 °C: $H_5 = 22.68$, $P < 0.001$; 30 °C: $H_4 = 18.45$, $P < 0.001$; 32 °C: $H_3 = 14.91$, $P < 0.001$). At 35 °C with water alone, the parasitism rate was only 40% parasitism on d 1 and 0% thereafter. When a 10% sugar solution was provided, female parasitoids had high parasitism activities (70–100%) for an extended period of time: 1–30 d at 20 °C, 1–20 d at 25 °C, and 1–15 d at 30 °C (Fig. 6B). Parasitism rates started to decrease after these periods and were significantly lower on d 40 at 20 °C, on d 30 at 25 °C, and on d 20 at 30 °C (20 °C: $H_8 = 30.64$, $P < 0.001$; 25 °C: $H_6 = 24.39$, $P < 0.001$; 30 °C: $H_4 = 18.04$, $P = 0.001$). Females reared with sugar solution at 32 and 35 °C exhibited a short period of efficient parasitism activities: 80% parasitism for 1–5 d at 32 °C and 25% parasitism for 1 d at 35 °C.

Discussion

Insect egg contents change from simple to complex forms during embryonic development (Vinson 2010). For most egg parasitoid wasps, the quality of host eggs declines with age, and beyond a certain embryonic stage is unable to support parasitoid development (Sousa and Spence 2001, Hirose et al. 2003, Vinson 2010). *Aprostocetus hagenowii* was found to face similar host age-related challenges in this study. By incubating *P. americana* oothecae at 20, 25, and 30 °C, we demonstrated that *A. hagenowii* could successfully develop in oothecae up to 50, 30, and 20 d old, respectively, representing 62–73% of total embryonic development time of *P. americana*. No parasitoid emerged from oothecae older than these ages. Among the oothecae ages with parasitoid emergence, *A. hagenowii* did not suffer any fitness penalty with increasing ootheca age. In contrast, several fitness traits (emergence, development time, body size, and longevity) of another competing solitary ootheca parasitoid (*Evania appendigaster* (L.)) were reported to decline with ootheca age (unpublished data). Compared to the solitary evaniid wasp, it is possible that the gregariousness of *A. hagenowii* allows faster consumption and utilization of cockroach eggs to occur before egg quality degrades. To overcome host age constraints, evaniid females are attracted to newly laid oothecae for oviposition. In contrast, *A. hagenowii* did not exhibit any ootheca age preference (Tee et al. 2011b). Because *A. hagenowii* females are smaller in size and more dependent on a carbohydrate food source for extended life span than *E. appendigaster* (Tee and Lee 2015), it would be costly for *A. hagenowii* to invest most of their time locating and selecting only young oothecae for oviposition. Our results suggest that efficient utilization of a wide ootheca age range without fitness costs (up to 72.9% total *P. americana* embryonic development) could be a critical trait for the reproductive success of *A. hagenowii*.

Temperature plays a vital role in defining the geographical and temporal distribution of insects. The temperature range between T_{lower} and T_{upper} for *A. hagenowii* was slightly lower (9.5–34.2 °C) but T_{opt} was slightly higher (31.1 °C) than those of *E. appendigaster* (range between T_{lower} and T_{upper}, T_{opt} for males and females = 12.7–36.1, 29.4 °C and 12.8–35.3, 28.8 °C, respectively; Bressan-Nascimento et al. 2010). Both parasitoid species had higher T_{lower} but a similar T_{upper} than the host, *P. americana* ($T_{lower} = 6.8$ °C and $T_{upper} = 36.0$ °C; Bressan-Nascimento et al. 2008, 2010). In subtropical regions, parasitoid populations may be suppressed during fall and winter seasons, which may have consequences for their efficacy

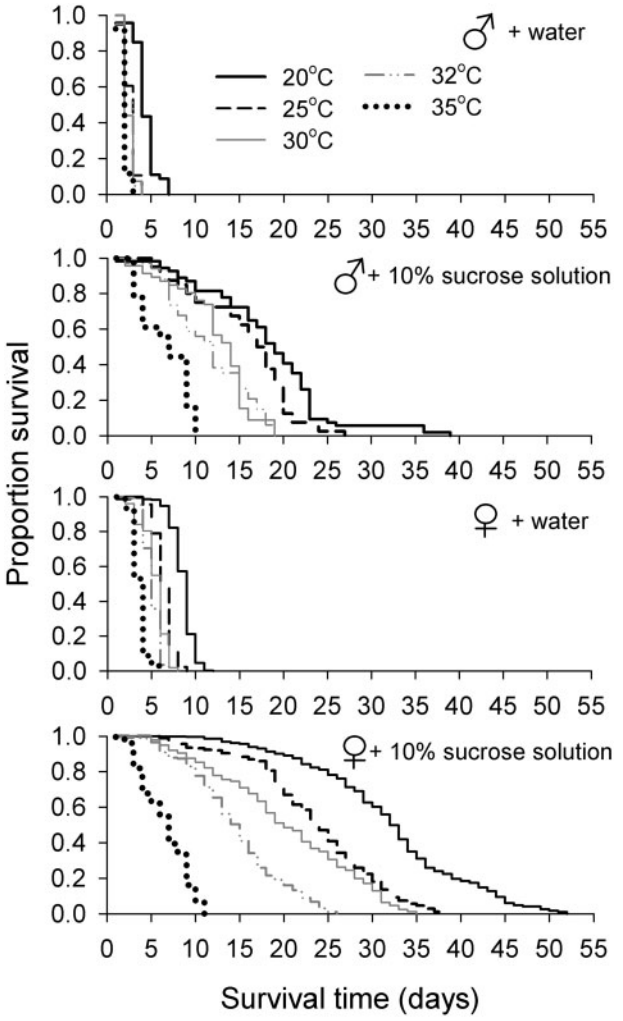

Fig. 4. Kaplan–Meier survival curves of *A. hagenowii* males and females provided with water alone or a 10% sucrose and held at 20–35 °C.

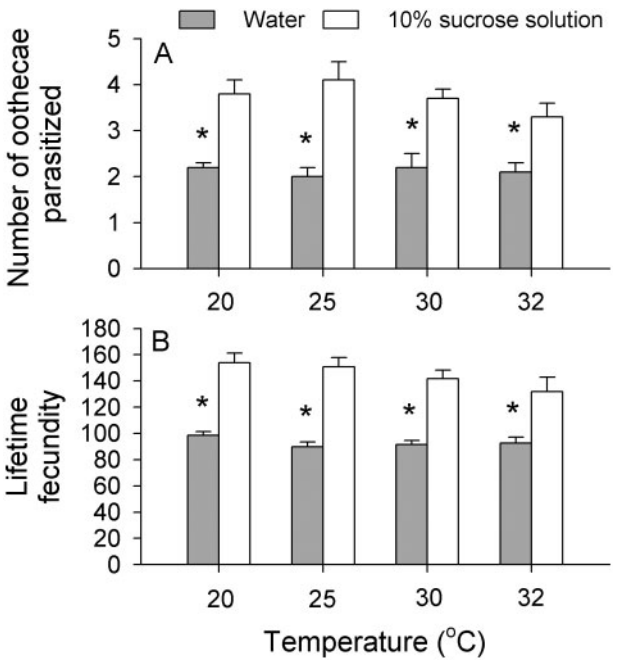

Fig. 5. The number of oothecae parasitized (**A**) and lifetime fecundity (**B**) of *A. hagenowii* females reared with water alone or a 10% sucrose solution at 20–32 °C. Asterisk (*) indicates significant difference between food treatments at each temperature (Student's *t*-test, $P < 0.05$). Vertical line on each bar indicates standard error of the mean ($n = 12$).

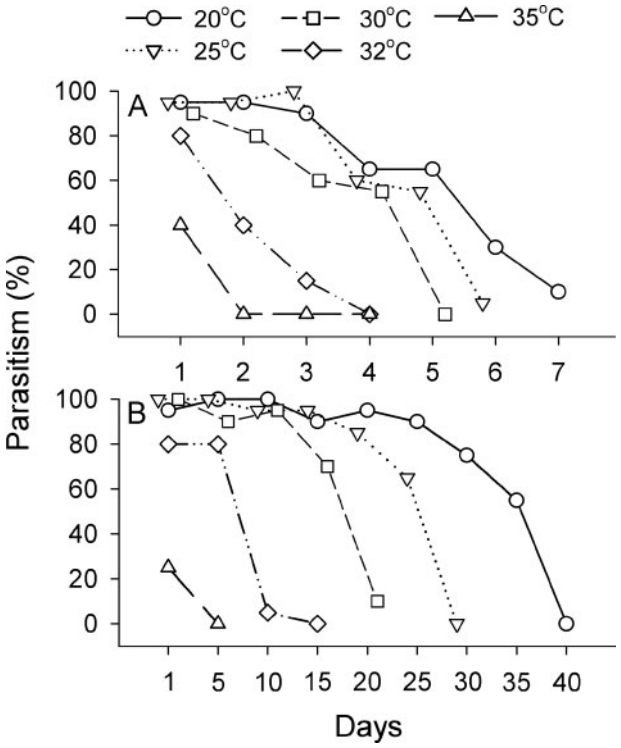

Fig. 6. Parasitism rate of oothecae (%) by *A. hagenowii* females at different time intervals when reared with water alone (**A**) or a 10% sucrose solution (**B**) at 20–35 °C.

in suppressing peak blattid populations during the following spring and summer seasons (Fleet et al. 1978, Suiter et al. 1998). Suiter et al. (1998) demonstrated that augmentative release of *A. hagenowii* is an effective way to enhance parasitism of *P. americana* oothecae around treehole habitats during warm seasons compared to naturally occurring parasitism activities in Florida.

Immature development of *A. hagenowii* at 20–32 °C took 34.0–71.0 d, which is similar to that documented by Cárcamo et al. (2013). Temperature (20–32 °C) did not affect the emergence rate, number of progeny/ootheca, and percentage female progeny of *A. hagenowii*. In contrast, *E. appendigaster* exhibited a wider developmental time range (35.8–94.7 d) at 20–30 °C, and its emergence and egg viability rates were influenced by temperature (Bressan-Nascimento et al. 2010). Thus, in comparison to *E. appendigaster*, immature *A. hagenowii* is less temperature sensitive and could be cultured under a wider rearing temperature range without compromising its quality as a biological control agent.

Temperature affected the survival and parasitism activities but not lifetime realized fecundity and number of oothecae parasitized by adult *A. hagenowii*. For female wasps, access to sucrose solution increased the number of progeny produced by 42–68% and number of oothecae parasitized by 57–100% compared to water alone (no sucrose solution). This finding demonstrates the importance of a carbohydrate food source in enhancing females' reproductive success. Males and females were short-lived (mean 2.0–4.4 and 5.0–9.2 d, respectively) when fed only water but had a significantly longer

life span when provided a sucrose solution (mean 11.6–18.3 and 14.5–32.0 d, respectively), except at 35 °C (< 7 d). At 32 °C, even though sugar-fed females could live up to 15 d, their efficient parasitism activities (≥ 80% parasitism) only lasted 5 d. Because 35 °C is lethal to the immatures and detrimental to adult survival, temperatures approaching this thermal threshold may affect females' parasitism over time, probably through rapid depletion of teneral reserves due to increased metabolism and dependency on water–sugar solution due to increased body water loss. At 20–30 °C, *A. hagenowii* females had considerably high parasitism activities (≥ 70% parasitism) over extended time periods, and it lasted for more days as temperature declined from 30 °C (15 d) to 20 °C (30 d). In contrast, *E. appendigaster* were most productive at 30 °C and less active at cooler temperatures of 17–25 °C (Bressan-Nascimento et al. 2010). This differing thermal response between competing species has been found in other parasitoids and may be responsible for their coexistence through temporal or niche differentiation (Sorribas et al. 2010, Le Lann et al. 2014).

By incubating *P. americana* oothecae at 20–30 °C, we demonstrated that *A. hagenowii* can successfully develop in oothecae for at least 72.9% of the total embryonic development time. For those parasitoids that successfully emerged, ootheca age did not influence their development time, number of progeny produced/ootheca, sex ratio, and body size, which differs from results reported for the ootheca parasitoid *E. appendigaster* (unpublished data). The temperature-dependent developmental threshold of *A. hagenowii* ranged from 9.5 to 34.2 °C and was optimal at 31.1 °C. These thresholds were close to those of *E. appendigaster* (Bressan-Nascimento et al. 2010). Temperature did not affect lifetime realized fecundity and number of oothecae parasitized by females but did influence adult survival and parasitism activities over time. A carbohydrate food source was critical for prolonging adult life span, but the period of efficient parasitism (≥80%) among sugar-fed females was still suppressed at ≥32 °C. Sugar-fed females sustained a prolonged period of efficient parasitism (≥70% for 15–30 d) at 20–30 °C, and it lasted longer at cooler temperatures.

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