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Cockroach Oothecal Parasitoid, *Evania appendigaster* (Hymenoptera: Evaniidae) Exhibits Oviposition Preference Towards Oothecal Age Most Vulnerable to Host Cannibalism

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Abstract

Many female parasitoid wasps optimize host selection to balance the benefits of high-quality hosts and the costs of predator- or hyperparasitoid-induced mortality risks to maximize their fitness. Cannibalism exists in many insect species and affects survival of parasitoid larvae developing in or on parasitized hosts. However, little is known about how parasitoid wasps resolve the fitness consequence of host cannibalism-induced mortality risk during host selection. We examined the effect of oothecal age on cannibalism in the American cockroach *Periplaneta americana* (L.) (Dictyoptera: Blattidae) and its effect on host age selection and fitness of its oothecal parasitoid *Evania appendigaster* (L.) (Hymenoptera: Evaniidae). *P. americana* differentially cannibalized 1-d-old (30–60%) versus 10- to 40-d-old oothecae (<9%). However, parasitoid females did not avoid but still preferred to parasitize 1-d-old (45%) over 10- to 40-d-old oothecae (1.6–20%). The parasitism rate was greater and the handling time was shorter on 1-d-old compared to older oothecae. For parasitoid progeny emerging from different-aged oothecae, regression analysis showed that development time increased and body size (measured as hind tibia length) and longevity decreased with oothecal age. These results demonstrate that reduced parasitoid progeny survival due to host cannibalism did not change the parasitoid's oviposition preference for newly laid oothecae, and that *E. appendigaster* females traded progeny survival for fitness gains for themselves and their progeny.

Key words: egg parasitoid, fitness trade-off, host age selection, host cannibalism, ootheca parasitoid

Parasitoid wasp larvae acquire all nutritional resources for development from a single host in or on which adult females have selected to lay eggs. Thus, the quality of the host, which varies with host species, size, and/or age, is, therefore, an important determinant of a parasitoid's fitness. Many parasitoid wasp females select high-quality hosts to maximize their fitness (Kouamé and Mackauer 1991, Ueno 1997, Chow and Mackauer 1999, Godin and Boivin 2000). However, hosts optimal for parasitoid development may also possess characteristics, or occur in locations, that make them vulnerable to attack by predators or hyperparasitoids, which would affect survival of parasitoid larvae developing on or inside the hosts. Consequently, predator- and hyperparasitoid-induced mortality and progeny fitness (due to host quality) are two natural selection pressures that help shape host foraging strategies. To overcome predator- or hyperparasitoid-induced mortality risk, female parasitoid wasps sometimes show adaptive host-foraging strategies, such as searching for low-risk or enemy-free host patches, if the benefits of dispersal outweigh mortality risks (Ayal and Green 1993, Höller et al. 1993, Taylor et al. 1998, Raymond et al. 2000) or attacking lower-quality hosts with a greater potential for overall profitability (Völkl and Kroupa 1997). In addition, parasitoid larvae may develop adaptations that reduce mortality risk, such as trading-off body size for fast development time or manipulating host behavior (Harvey and Strand 2002, Grosman et al. 2008).

Another mortality risk facing parasitoids is host cannibalism, whereby parasitized hosts are either selectively or randomly cannibalized by conspecifics (Anderson and Solbreck 1992, Reed et al. 1996, Wang and Daane 2004). In a modeling study of the host-parasitoid interaction, Rudolf et al. (2012) reported that selective host cannibalism affected population dynamics of both the host

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and the parasitoid and altered host selection strategies of the parasitoid. Although cannibalism is prevalent among many insect species (Richardson et al. 2010), studies of the impact of host cannibalism-induced mortality on parasitoid fitness and host selection strategies are scarce.

In this study, we investigated the influence of cannibalism of oothecae (bean-shaped egg cases that enclose 12–16 cockroach eggs; Bell and Adiyodi 1981) of the American cockroach, *Periplaneta americana* (L.) (Dictyoptera: Blattidae), on host selection behavior of the ootheca parasitoid *Evania appendigaster* (L.) (Hymenoptera: Evaniidae). *E. appendigaster* is a solitary egg parasitoid wasp that commonly lays an egg inside an ootheca (Bressan-Nascimento et al. 2010). All cockroach eggs are consumed by the parasitoid larva before it chews an exit hole through the ootheca shell to emerge as an adult wasp. *P. americana* is a cosmopolitan major pest cockroach of medical and economic importance (Rust 2008). Adult female cockroaches produce an average of 1–2 oothecae weekly for their entire lifespan (Gould and Deay 1938, Roth and Willis 1956). The ootheca is relatively soft and reddish-brown in color upon being laid, and it hardens and darkens during the first few days of incubation. Oothecal cannibalism is frequently observed in *P. americana* (Gould and Deay 1938, Roth and Willis 1960), but whether the oothecal shell-hardening process with age affects cannibalism risk is poorly understood. On the other hand, insect eggs are a dynamic nutrient pool, and the quantity and quality of this resource available for immature parasitoid development change with age (Vinson 2010). Many female egg parasitoids have been shown to select the optimal host age for oviposition because host age affects the fitness traits of ovipositing females (e.g., handling time, success rate of parasitism) and their progeny (e.g., development time, body size, fecundity, longevity) (Huis et al. 1991, Godin and Boivin 2000, Hirose et al. 2003, Zhang et al. 2014).

In this study, we expected that *E. appendigaster* females would consider host cannibalism-induced mortality risk into their host-age selection behavior to maximize their fitness. We hypothesized that different-aged oothecae would vary in their vulnerability to cannibalism by cockroaches. Specifically, we expected young oothecae with a relatively softer shell to be more vulnerable to cannibalism than older oothecae. Regarding parasitism, we envisioned two possible scenarios. *E. appendigaster* females could avoid ovipositing into these high-risk oothecae to reduce progeny mortality, as oviposition into less vulnerable oothecae could provide a greater fitness return. Alternatively, evaniid females could accept those vulnerable oothecae for oviposition if they could optimize host utilization through a trade-off between progeny survival and their own fitness and that of their progeny.

We first assessed the vulnerability of oothecae to cannibalism by *P. americana* in relation to oothecal ages (1, 10, 20, 30, and 40 d old), cockroach life stages (mid-nymphs, adult males and females) and oothecal parasitism status (unparasitized and *E. appendigaster*-parasitized). Oothecal age preference in *E. appendigaster* was examined using a multiple-choice method, in which the five age classes of oothecae were simultaneously presented to a female parasitoid for parasitism. We also assessed the influence of oothecal age on various fitness traits of *E. appendigaster* females (handling time, success rate of parasitism, and progeny sex ratio) and their progeny [development time, body size measured as hind tibia length (HTL in mm), longevity, and lifetime realized fecundity] using regression analyses.

Materials and Methods

Cockroach and Parasitoid Cultures

The starting cultures of *P. americana* and *E. appendigaster* were originally collected using glass jar traps and sentinel oothecae in Penang, Malaysia and established at the Urban Entomology Laboratory, Universiti Sains Malaysia in 1997 and 2007, respectively. These insect cultures were maintained using the procedures described by Tee and Lee (2013). Insect cultures and experiments were maintained at $26.1 \pm 0.1°C$, $53.3 \pm 6.4\%$ RH, and a photoperiod of 12:12 (L:D) h. For use in the experiments, 1–2-d-old *E. appendigaster* males and females that emerged from parasitized oothecae were either kept separated as naive individuals or paired to obtain mated females. To provide oothecae for experiments, styrofoam pieces ($15 \times 30 \times 3$ cm) were placed in *P. americana* rearing tanks ($45 \times 30 \times 30$ cm, each contained 100–120 adult females and 10 adult males) (Yeh 1995) from 1700 to 0800 h; newly laid oothecae (<24 h) were collected daily from these pieces of styrofoam.

Cannibalism of unparasitized and parasitized oothecae of different ages by *P. americana*

In this experiment, we studied the influence of oothecal age (1, 10, 20, 30, and 40 d old), cockroach life stage [male, female, and mid-nymph (2.0–2.5 cm in body length)], and ootheca parasitism status (unparasitized and *E. appendigaster*-parasitized) on oothecal cannibalism by *P. americana*. This experiment was 5 (oothecal age) × 3 (life stage) × 2 (parasitism status) factorial. The five oothecal age classes were selected based on the average incubation period of 46 d documented for *P. americana* at 26°C in a preliminary test.

Newly laid oothecae were aged for 1, 10, 20, 30, and 40 d to provide the unparasitized oothecae. To obtain *E. appendigaster*-parasitized oothecae of similar ages, newly laid, 9, 19, 29, and 39-d-old oothecae were individually subjected to parasitism by a mated *E. appendigaster* female inside a Petri dish (9.0 cm diameter, 1.5 cm height) for 24 h. After 24 h, exposed oothecae were removed and inspected under a stereomicroscope (EZ Vision, Saxon, Guangzhou, China) to determine whether parasitoids had attempted to parasitize them. We could identify the ootheca on which *E. appendigaster* had attempted oviposition by the presence of a dried yellowish fluid that oozed out of the ovipositor puncture wound on the surface. These oothecae were selected and assigned as parasitized oothecae.

The test arena consisted of a tray ($50 \times 37 \times 9$ cm) that contained a triangle-shaped cardboard harborage (20×3 cm), a water source, and dry cat food. A group of 10 *P. americana* males, females, or nymphs were introduced into a test arena and allowed to acclimatize for 48 h. After 48 h, females carrying an ootheca were replaced with a new one because feeding activity is minimal during this gravid stage (Rollo 1984). For each cockroach life stage/sex, 10 oothecae from one of the combinations of ootheca age and parasitism status were introduced into the test arena. After 48 h, we recorded the number of oothecae that had been cannibalized. Oothecae for which only the keel was eaten (>50% of the keel) were also classified as being cannibalized because eggs within these oothecae frequently failed to hatch due to rapid water loss and microbial invasion (Roth and Willis 1955, Bell and Adiyodi 1981). Six replicates were performed for each combination of oothecal age, oothecal parasitism status, and cockroach life stage.

Oothecal age preference of *E. appendigaster*

The sequence of oviposition behavior of an *E. appendigaster* female on an ootheca is as follows: 1) host contact and probing using antennae; 2) unsheathe ovipositor to probe and finding a suitable drilling site; 3) female remains quiescent with tip of ovipositor touching the surface of the ootheca; 4) drilling and insertion of ovipositor; 5) oviposition; 6) withdrawal of ovipositor, leaving the ootheca (Yeh and Mu 1994). In this section and the following experiments, an ootheca was considered to have been selected as a host if an *E. appendigaster* female performed this sequence of oviposition behavior.

Oothecal age preference was studied using a multiple-choice test in which the age of the ootheca that females first attempted to parasitize was determined and defined as the most preferable age for oviposition. The test arena consisted of a 9 cm diameter Petri dish (1.5 cm height) containing a fitted piece of filter paper (9 cm diameter). A 5-cm circle was drawn on the center of the filter paper, and five oothecae (1, 10, 20, 30, and 40 d old) were randomly placed at five points at equal distance from one to another along the circumference of the circle. The Petri dish was capped with a lid that was perforated with a 5-cm hole at the center. A mated female *E. appendigaster* was placed on top of the Petri dish inside an inverted capped-round plastic container (7 cm diameter, 4 cm height). The female parasitoid was allowed to acclimatize within the container for 30 min before the cap was removed to allow free entry into the Petri dish. The age of the first ootheca on which the female showed oviposition behavior was recorded. This experiment was replicated for 60 mated female wasps.

Impact of ootheca age on handling time and fitness traits of *E. appendigaster* on different-aged oothecae

Oothecal handling time was studied using a no-choice method. One mated and oviposition-inexperienced female was introduced into a 9 cm Petri dish (1.5 cm height) containing an ootheca from one of the five age classes (1, 10, 20, 30, and 40 d old). The Petri dish was covered with a lid, and the time from which the female parasitoid first contacted the ootheca to withdrawal of its ovipositor was recorded. For each oothecal age class, experiment was repeated until 12 females (replicates) were observed to complete an oviposition event.

Fitness traits of *E. appendigaster* females (percentage parasitism attempted, percentage successful parasitism, and progeny sex ratio) and their progeny (development time, body size, longevity, and lifetime realized fecundity) on 1, 10, 20, 30, and 40-d-old oothecae also were assessed using no-choice experiments. For each age class, 30 oothecae were individually exposed to parasitism by a mated and oviposition inexperienced *E. appendigaster* female inside a 9 cm diameter Petri dish. After 24 h, exposed oothecae were removed and observed under a stereomicroscope for the presence of an oviposition puncture to determine whether parasitoids had attempted to parasitize them. Oothecae then were individually transferred into a 2 ml microtube with a perforated lid and checked daily for parasitoid emergence. Parasitoids that had emerged were sexed and reared individually inside a plastic container (100 ml) with a screened lid and a 10% honey solution (delivered in a cotton-plugged 2 ml microtube). Parasitoid survival was checked daily to determine their longevity. Upon their death, parasitoids were observed under an SZ61 stereomicroscope (Olympus, Tokyo, Japan) equipped with IC Imaging Standard, version 2.1 (The Imaging Source Europe GmbH, Bremen, Germany), and their right hind tibia (in mm) was measured, as an indicator of body size, using Analysis Image Processing software (Soft Imaging System GmbH, Münster, Germany). In addition, starting from the day of emergence, female parasitoids were 1) paired with a naive male (from parasitoid culture), and the male was removed after they were observed to have mated, and 2) presented with two 1–2-d-old oothecae every 24 h to test for oviposition. Oothecae were removed after 24 h exposure and transferred individually into a 2 ml microtube with a perforated lid. Parasitoid emergence from these exposed oothecae was checked daily and recorded to determine lifetime realized fecundity for each female progeny. In this

experiment, we calculated the proportion of parasitism attempted, the proportion of successful parasitism, and progeny sex ratio (proportion of males) of *E. appendigaster* on oothecae of different ages. For parasitoids that had emerged, we recorded their development time, longevity, HTL, and lifetime realized fecundity (number of progeny emerged).

Data analysis

Percentage cannibalism data were arcsine square-root-transformed to normalize their distribution (Conover and Iman 1981) and were then analyzed using three-way analysis of variance (ANOVA) with oothecal age class, cockroach life stage, and oothecal parasitism status as main effects. Because no significant effect of the interactions was detected (see Results), we used independent one-way ANOVA to analyze the effect of each significant main factor, followed by separation of means using Tamhane's test (because unequal variance was detected using Levene's test) (Zar 1999). Percentages of oviposition among oothecal age classes were analyzed using the chi-square test. When a significant difference among oothecal age classes was detected, we used a Tukey-type multiple comparison test for posthoc analysis (Zar 1999). Handling times, development time, HTL, longevity, and lifetime realized fecundity data were regressed against ootheca age using linear (logarithmic) and nonlinear (quadratic) models. When a significant relationship was established for both models, we used Akaike's Information Criterion (AIC) to determine which model (linear or nonlinear) better described the relationship. AIC was calculated using the formula $AIC = n\ln(SSE/n) + 2p$, where n is the number of observations, p is the number of parameters in the model, and SSE is the sum of squared error. The model with the lower AIC value is the better fit for the recorded data (Akaike 1974). Proportion parasitism attempted and proportion successful parasitisms were analyzed using binomial logistic regression with parasitism status (0 = not attempted, 1 = attempted) and parasitism success (0 = not success, 1 = success) as a dependent variable, respectively, and oothecal age as a predictor variable (Wilson and Hardy 2002). All statistical analyses were performed using SPSS version 20 (IBM Corp, New York, NY), and level of significant was set at $\alpha = 0.05$.
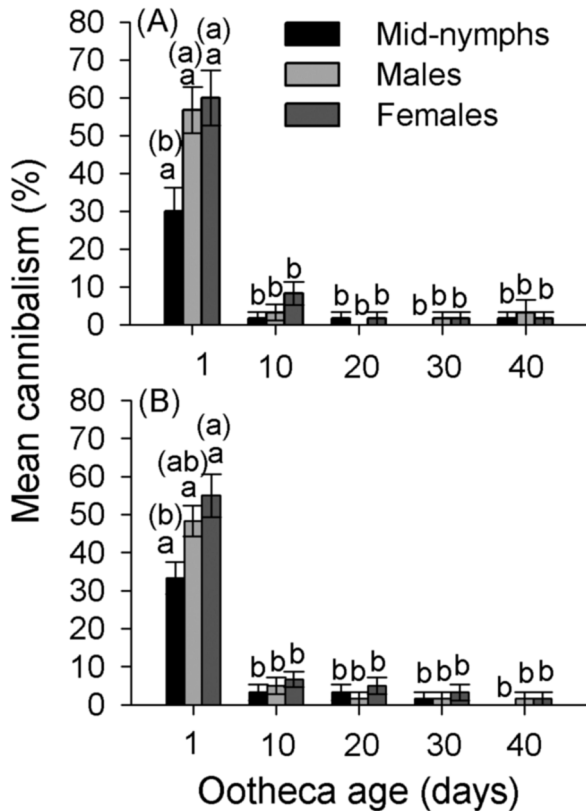
Results

Cannibalism of unparasitized and parasitized oothecae of different ages by *P. americana*

Percentage cannibalism of oothecae was significantly influenced by oothecal age and cockroach life stage. Ootheca parasitism status and any interaction between and among main effects did not significantly affect percentage cannibalism (Table 1; Fig. 1). In all combinations of parasitism status and cockroach stage, oothecal age significantly affected the occurrence rate of cannibalism (unparasitized oothecae, nymphs: $F_{4, 25} = 20.23$, $P < 0.001$; males: $F_{4, 25} = 36.46$, $P < 0.001$; females: $F_{4, 25} = 30.95$, $P < 0.001$; *E. appendigaster*-parasitized oothecae, nymphs: $F_{4, 25} = 20.95$ $P < 0.001$; males: $F_{4, 25} = 31.38$, $P < 0.001$; females: $F_{4, 25} = 24.76$, $P < 0.001$). Posthoc analysis revealed that 1-d-old oothecae experienced a higher cannibalism pressure (30.0–60.0%) than all the other age classes (10- to 40-d-old oothecae; 0–8.3%; Fig. 1). Differences in oothecal cannibalism rates between cockroach life stages were found only in 1-d-old oothecae, where nymphs cannibalized a lower percentage of unparasitized oothecae than males and females ($F_{2, 15} = 6.09$, $P = 0.012$; Fig. 1A) and a lower percentage of *E. appendigaster*-parasitized oothecae than females ($F_{2, 15} = 5.62$, $P = 0.015$; Fig. 1B).

Table 1. Results of three-way ANOVA of the effects of ootheca age, cockroach life stage, and ootheca parasitism status on percentage cannibalism of oothecae in *P. americana*

| Source	F	df	P
Ootheca age	161.08	4	< 0.001
Life stage	7.46	2	0.001
Ootheca parasitism status	0.47	1	0.493
Ootheca age × life stage	1.95	8	0.056
Ootheca age × ootheca parasitism status	0.84	4	0.5
Life stage × ootheca parasitism status	0.17	2	0.847
Ootheca age × life stage × ootheca parasitism status	0.28	8	0.97

Fig. 1. Mean (± SE) percentage cannibalism of different-aged (A) unparasitized and (B) *E. appendigaster*-parasitized oothecae by *P. americana* mid-nymphs, males, and females. Letters without and with parentheses indicate significant differences in percentage cannibalism among different-aged oothecae by each cockroach life stage and among cockroach life stages in each ootheca age, respectively (Tamhane's test, $P < 0.05$). The vertical line on each bar represents standard error of the mean ($n = 6$).

Oothecal age preference by *E. appendigaster*

Oothecal age significantly influenced oviposition preference by *E. appendigaster* ($\chi^2_4 = 29.5$, $P < 0.001$; Fig. 2) in the multiple-choice experiments. Females preferred 1-d-old oothecae (45.0%) for oviposition, followed by 10- to 30-d-old oothecae (16.7–20.0%) and 40-d-old oothecae (1.6%).

Impact of ootheca age on handling time and fitness traits of *E. appendigaster* on different-aged oothecae

Evaniid females spent an average of 1,135 s of handling time on 1-d-old oothecae, 1,663 s on 10-d-old oothecae, and 1,956–2,341 s
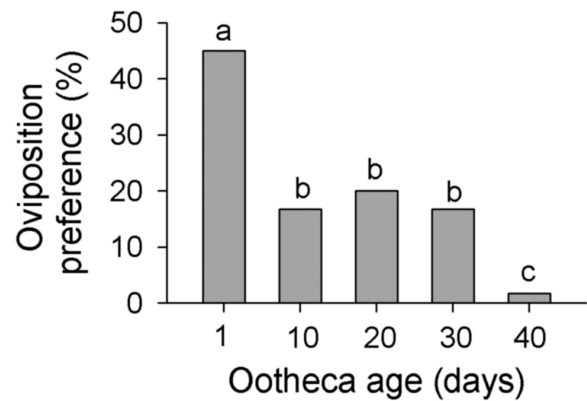
Fig. 2. Oviposition preference (%) of *E. appendigaster* females ($n = 60$) when presented simultaneously with five oothecae each at 1, 10, 20, 30, and 40 d old. Bars labeled with different letters are significantly different (chi-square test followed by Tukey-type multiple comparisons, $P < 0.05$).

on 20–40-d-old oothecae. The nonlinear model ($F_{2,57} = 21.93$, $r^2 = 0.435$, $P < 0.001$, AIC = 404.6) was found to better illustrate the relationship between handling time and oothecal age than the linear model ($F_{1,58} = 40.62$, $r^2 = 0.412$, $P < 0.001$, AIC = 602.2; Fig. 3A).

Logistic regression analyses show that ootheca age significantly affected the proportion of successful parasitism ($\chi^2_1 = 22.89$, $P < 0.001$; Fig. 3C) but not the proportion of parasitism attempted ($\chi^2_1 = 3.87$, $P = 0.05$; Fig. 3B) and sex ratio ($\chi^2_1 = 0.789$, $P = 0.374$; Fig. 3D) of *E. appendigaster* on different-aged oothecae. Observed successful parasitism (ootheca from which a wasp emerged) by evaniid females on 1- and 10-d-old oothecae was 0.87 and 0.77, respectively. Lower proportions were documented for 20- and 30-d-old oothecae (0.53 and 0.33, respectively). No parasitoid emerged from 40-d-old oothecae. Logistic regression analysis estimated that the probability of successful parasitism decreased with ootheca age ($\chi^2_1 = 22.89$, $P = < 0.001$; Fig. 3C). Of those progeny that emerged, their sex ratio ranged from 0.35 to 0.50 among 1- to 30-d-old oothecae.

Development times of males and females increased from 44.9 to 48.0 d and 45.8 to 48.6 d, respectively, as oothecal age increased from 1 to 30 d old (Fig. 4A). The relationship between development time and oothecal age in both sexes was better described by the linear model (males: $F_{1,29} = 30.77$, $r^2 = 0.515$, $P < 0.001$, AIC = 5.72; females: $F_{1,42} = 36.25$, $r^2 = 0.463$, $P < 0.001$, AIC = 5.87) than the nonlinear model (males: $F_{2,28} = 16.59$, $r^2 = 0.542$, $P < 0.001$, AIC = 5.91; females: $F_{2,41} = 17.73$, $r^2 = 0.0464$, $P < 0.001$, AIC = 7.83).

Males and females that emerged from older oothecae had shorter HTL values than those that developed in younger oothecae (Fig. 4B). The relationship between HTL and oothecal age in males (3.25–3.63 mm) and females (3.10–3.57 mm) (was better fitted to the linear model (males: $F_{1,29} = 12.36$, $r^2 = 0.299$, $P = 0.002$, AIC = −124.27; females: $F_{1,42} = 20.22$, $r^2 = 0.325$, $P < 0.001$, AIC = −182.95) than to the nonlinear model (males: $F_{2,28} = 6.08$, $r^2 = 0.302$, $P = 0.006$, AIC = −122.45; females: $F_{2,41} = 9.07$, $r^2 = 0.348$, $P = 0.001$, AIC = −182.42).

Longevity of males and females decreased from 31.6 to 24.2 d and 22.4 to 16.4 d, respectively, as the oothecal age increased from 1 to 30 d old (Fig. 4C). The relationship between longevity and oothecal age in males and females was better explained by the linear (males: $F_{1,29} = 12.95$, $r^2 = 0.309$, $P = 0.001$, AIC = 89.06; females: $F_{1,42} = 9.56$, $r^2 = 0.185$, $P = 0.004$, AIC = 113.11) than the nonlinear model (males: $F_{2,28} = 7.43$, $r^2 = 0.346$, $P = 0.003$, AIC = 89.32; females: $F_{2,41} = 4.68$, $r^2 = 0.185$, $P = 0.015$, AIC = 115.11).
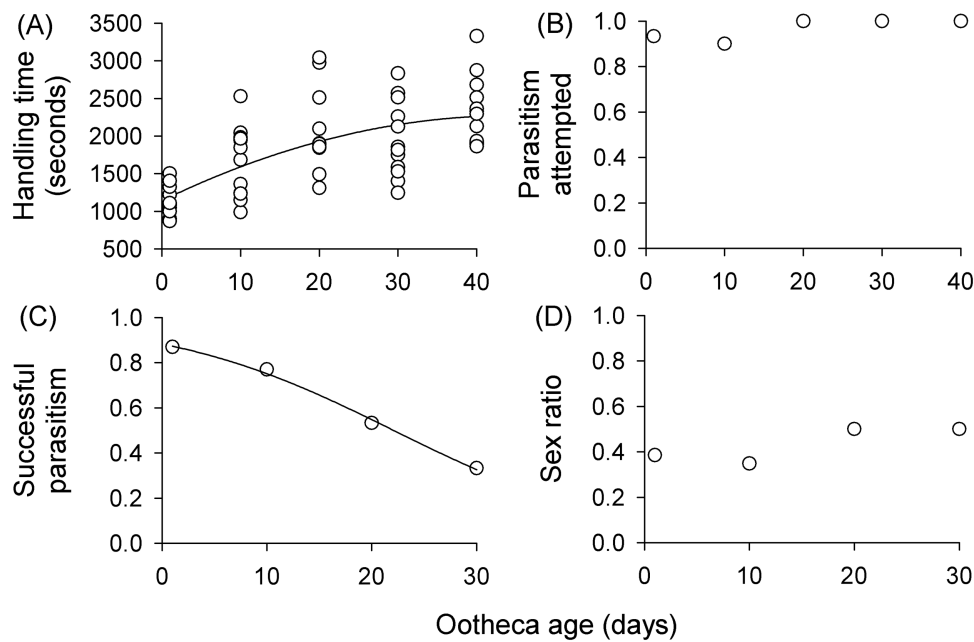
Fig. 3. Relationship between ootheca age and various fitness traits of *E. appendigaster*: (A) handling time ($y = 1{,}140.5 + 50.75x - 0.5684x^2$, $F_{2, 57} = 30.77$, $r^2 = 0.0435$, $n = 12$ for each ootheca age, $P < 0.001$); (B) proportion parasitism attempted; (C) proportion successful parasitism; and (D) sex ratio. In (B), (C), and (D), binary data were analyzed using logistic regressions ($n = 120$, 120, and 75, respectively) but are presented as a proportion for each ootheca age. A significant relationship was established only in (C), in which the fitted logistic regression line shows the estimated probability (P) of an ootheca successfully producing a wasp ($P = \exp(2.013 - 0.019x) / (1 + \exp(2.013 - 0.019x))$, $x =$ ootheca age).

Lifetime realized fecundity of female parasitoids varied between 15.2 and 17.6 progenies produced (Fig. 4D). No significant relationship was found between lifetime realized fecundity and oothecal age (linear: $F_{1, 42} = 0.222$, $r^2 = 0.005$, $P = 0.6401$; nonlinear: $F_{2, 41} = 0.609$, $r^2 = 0.029$, $P = 0.549$).

Discussion

Our results show that cannibalism of oothecae was not random; 1-d-old oothecae were more vulnerable to cannibalism by all three life stages of *P. americana* (mid-nymphs, males, and females) than older oothecae (10–40 d old). Cannibalism can be both beneficial and costly for *P. americana* populations. Cockroaches can obtain and recycle essential nitrogenous resources back into cockroach aggregation through cannibalism (Cochran 1985, Ross and Mullins 1995, Nalepa and Bell 1997, Bell et al. 2007). Nalepa and Mullins (1992) estimated that adult female wood cockroach, *Cryptocercus punctulatus* Scudder, could recover up to 58.7% of the nitrogen she invested into her brood inside a single ootheca by eating the oothecal shell after hatch. Due to the nutritional richness of oothecae (12–16 yolk-rich eggs enclosed inside a protein-rich oothecal shell) (Bell and Adiyodi 1981, Kramer et al. 1991), cannibalism of oothecae is expected to increase the fitness of cannibals by increasing growth rate, fecundity, longevity, and energy sources, as demonstrated in other insect species that eat conspecific eggs (Gagné et al. 2002, Richardson 2010). Oothecae within a cockroach population are expected to experience high cannibalism risk if a wide range of oothecal ages is being indiscriminately cannibalized (as a result of a long time window of vulnerability). This would greatly reduce the numbers of nymphs and adults (e.g., in a cyclic or chaotic pattern), as demonstrated in population dynamics studies of other cannibalistic insects (e.g., *Tribolium* and *Plodia*) (Costantino et al. 1997, Briggs et al. 2000, Claessen et al. 2004), thereby leading to cockroach populations consistently experiencing the adverse effects of reduced size

and unstable age-class distribution (Polis 1981). Established populations of blattid pest cockroaches are known to consist of overlapping generations and were characterized by the persistence of a moderate-to-high proportion of nymphs (0.47–0.72) (Fleet et al. 1978, Appel and Rust 1985, Tee et al. 2011). This group-living lifestyle could enhance the fitness of group members by increasing survival, growth, mating opportunities, and foraging efficiency for shelter and food sources (reviewed in Bell et al. 2007, Lihoreau et al. 2012). Reduction in population numbers due to intense cannibalism likely is a trade-off with the benefits associated with group living. In contrast, differential cannibalism of only newly laid oothecae may minimize the effect of population size reduction and oscillation because the time window of vulnerability is shortened and the age gap between oothecae is minimized (*P. americana* females produce an average of 1–2 oothecae per week in their entire lifespan) (Gould and Deay 1938, Roth and Willis 1956). Therefore, our results suggest that differential cannibalism of newly laid oothecae may be adaptive for *P. americana* to balance the costs and benefits of group living.

Despite the mortality risk associated with differential cannibalistic behavior of *P. americana*, we found that *E. appendigaster* females did not avoid but rather preferred to parasitize 1-d-old oothecae. Our results show that oothecal age had a positive and negative linear relationship with development time and body size of the parasitoid, respectively, indicating that the fitness costs of longer development time and smaller body size were intensified with oothecal age. Development time and body size are important fitness traits for parasitoid wasps. Development time determines the amount of time available to exploit mates and host patches before arrival of other competitors, the intrinsic rate of increase of a parasitoid population (Sequeira and Mackauer 1994), and the time window of vulnerability to hyperparasitism and predation (Harvey and Strand 2002). Body size is a strong indicator of several parasitoid fitness traits, such as survival, reproduction, and flight ability (Ellers et al. 1998, Boivin and Martel 2012). This host age-specific constraint
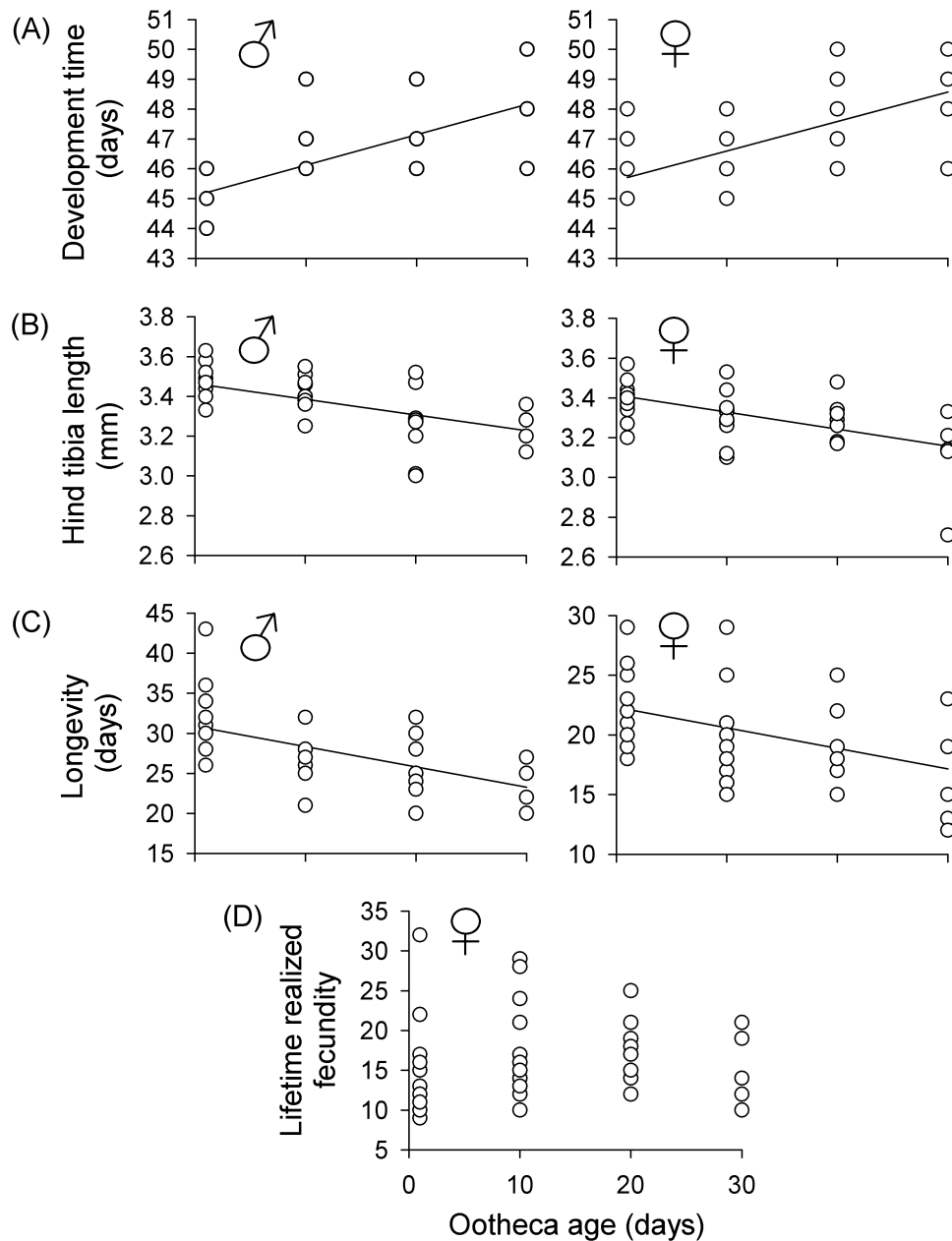
Fig. 4. Relationship between ootheca age and various fitness traits of *E. appendigaster* progeny: (A) development time of males ($y = 0.102x + 45.10$, $F_{1, 29} = 30.77$, $r^2 = 0.515$, $P < 0.001$) and females ($y = 0.099x + 45.61$, $F_{1, 42} = 36.25$, $r^2 = 0.463$, $P < 0.001$); (B) hind tibia length of males ($y = -0.008x + 3.47$, $F_{1, 29} = 12.36$, $r^2 = 0.299$, $P = 0.002$) and females ($y = -0.009x + 3.42$, $F_{1, 42} = 20.22$, $r^2 = 0.325$, $P < 0.001$); (C) longevity of males ($y = -0.254x + 30.88$, $F_{1, 29} = 12.95$, $r^2 = 0.309$, $P = 0.001$) and females ($y = -0.172x + 22.31$, $F_{1, 42} = 9.56$, $r^2 = 0.185$, $P = 0.004$); and (D) lifetime realized fecundity ($P > 0.05$ for both linear and nonlinear models). Sample size for oothecae aged 1, 10, 20, and 30 d old: males = 10, 8, 8, and 5; females = 16, 15, 8, and 5, respectively.

on development time and body size is unique among egg and pupal idiobiont parasitoids. In this relationship, the host size/resource is relatively fixed across a range of host ages, but is comparatively less suitable for parasitoid development as the host ages. Parasitoid larvae may take longer to develop and have difficulties in consuming host resources and assimilating sufficient nutrients from well-developed embryos or well-sclerotized pupae (Otto and Mackauer 1998, Hirose et al. 2003). However, idiobiont larval parasitoids can trade long development time for increased body size (and other fitness correlates) in older larvae due to larger host resources (Wei et al. 2014). On the other hand, koinobiont larval parasitoids have been found to exhibit developmental plasticity in response to mortality

risk associated with host feeding environments (Harvey and Strand 2002); for example, parasitoids that utilize hosts living in a low mortality risk environment (e.g., concealed area) can extend their development time in smaller-sized hosts to benefit from the greater nutrient pool accumulated in later host stages, whereas immature parasitoids utilizing hosts in a high-risk environment (e.g., exposed areas) can shorten their development time at the costs of potential gains in body size during development in hosts of different ages and sizes (Harvey and Strand 2002).

Most solitary parasitoid wasps are known to allocate female progeny in hosts of higher quality (young or large size) and male progeny in hosts of lower quality (old or small size) (King 1994,

Ueno 1998, Sousa and Spence 2001). This scenario is in agreement with the host quality model that predicts that the fitness return is greater for being a large female than a large male (Charnov et al. 1981, King 1990, Ueno 1999). Although evaniid females developing in older oothecae were small in size, their fecundity was unaffected by ootheca age. This may partly explain why they did not adjust the progeny sex ratio in response to oothecal age according to host quality model. In this study, only one ootheca was presented to each evaniid female in the sex-ratio experiment. Studies of other solitary parasitoid wasps have shown that upon encountering hosts, females may judge their quality either relative to those simultaneously presented or to those previously experienced to determine the sex of progeny they want to lay (King 1994, Ode and Heinz 2002). Our study design could not rule out the possibility of progeny sex manipulation in *E. appendigaster* in response to oothecal age.

Although female fecundity was unaffected by oothecal age, their longevity declined with oothecal age. This detrimental effect of host age may indirectly affect female fecundity in the natural environment. Visser et al. (2010) reported that most parasitoid wasps cannot undergo lipogenesis and must accumulate essential lipid reserves from the host during larval development. Lipid reserves are primarily used by adult parasitoid wasps for egg production and, when carbohydrate-rich food sources are scarce, for body maintenance and locomotion (Ellers and van Alphen 1997, Rivero and West 2002, Giron and Casas 2003). Therefore, smaller sized and shorter-lived females produced from older-aged oothecae in the field likely face the dilemma of whether to allocate lipid reserves for body maintenance or egg production.

E. appendigaster progeny incurred multiple fitness costs (development time, body size, longevity) with increasing age of the oothecae in which they developed. Thus, unlike several egg parasitoid species from the families Trichogrammatidae and Scelionidae, both females and immature stages seem to lack the ability to manipulate host embryos (e.g., breakdown host tissue and arrest host development) to optimally suit their development needs without incurring reduced fitness (Strand et al. 1986, Vinson 1998, Pennacchio and Strand 2006, Zhou et al. 2014). One way to counteract these host-age induced development constraints on progeny is for female parasitoid wasps to optimize progeny fitness by locating and ovipositing in young hosts (Vinson 1998). In this study, *E. appendigaster* females preferred to attack 1-d-old oothecae over older oothecae, even though this would expose their progeny to the mortality risk of host cannibalism. However, we found that evaniid females produced higher numbers of progeny (higher successful parasitism rate) on 1-d-old compared with older oothecae, which might offset the progeny mortality risk due to host cannibalism. Females also gained more opportunity time if they chose 1-d-old oothecae for parasitism, as handling time increased curvilinearly with oothecal age. Thus, it may be adaptive for evaniid females to trade progeny survival for greater fitness benefits for themselves (higher successful parasitism rate and shorter handling time) and their progeny (multiple fitness gains).

In conclusion, we demonstrate that 1-d-old oothecae were highly vulnerable to cannibalism by *P. americana* adults and nymphs. Cannibalism of only newly laid oothecae is likely to allow a cockroach population to profit from eating conspecific eggs without triggering population oscillation (e.g., reduction in size and unstable age class distribution). *E. appendigaster* females did not avoid but rather preferred to parasitize newly laid oothecae, even though their progeny would be at risk of being accidentally eaten by cockroaches. In comparison to newly laid oothecae, parasitoid progeny developing in older oothecae incurred multiple fitness costs, including increased development time and reduced body size and longevity.

Adult females also had lower successful parasitism rates and longer host handling times on older oothecae. These findings suggest that *E. appendigaster* females balance host cannibalism-induced mortality risk with fitness gains for themselves and their progeny by selecting younger oothecae to parasitize.

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