

Water balance profiles, humidity preference and survival of two sympatric cockroach egg parasitoids *Evania appendigaster* and *Aprostocetus hagenowii* (Hymenoptera: Evaniidae; Eulophidae)



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ABSTRACT

The impact of desiccation on habitat selection, foraging and survival has been characterized for many insects. However, limited information is available for parasitic wasps. In this study, water balance, relative humidity (RH) preference, and effect of humidity on survival of solitary *Evania appendigaster* (L.) (Hymenoptera: Evaniidae) and gregarious *Aprostocetus hagenowii* (Ratzeburg) (Hymenoptera: Eulophidae) were examined. These species are both oothecal parasitoids of the American cockroach *Periplaneta americana* (L.) (Dictyoptera: Blattellidae). *E. appendigaster* had significantly higher cuticular permeability (CP) and a lower surface area to volume ratio but a similar percentage of total body water content compared to *A. hagenowii*. No differences in these attributes were found between sexes of each parasitoid species. The percentage of total body water loss rates among *E. appendigaster* males and females and *A. hagenowii* females were similar but significantly lower than that of *A. hagenowii* males. All parasitoids except *E. appendigaster* males exhibited reduced survival times as the RH of their enclosure decreased from 87% to 38%, but this phenomenon did not occur when parasitoids were given a sugar solution. In environmental chambers with a 44–87% RH gradient, both sexes of *E. appendigaster* resided significantly more often in the 87% RH chamber than in the 44% RH chamber. For *A. hagenowii*, females preferred both the driest and the wettest chambers and males preferred the driest ones. These results demonstrate the water balance profile and its relationship to life history traits and differential responses to RH in these competing parasitoid wasps, suggesting the role of physiological and behavioral adaptations in shaping their ecological niche.

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1. Introduction

Terrestrial insects are susceptible to desiccation due to their small body size and large body surface area to volume ratio, as these features allow a great portion of their total body water (TBW) to be lost via evaporation at the cuticle surface (Hadley, 1994; Schilman et al., 2007). To overcome the desiccation stress imposed by their habitats, insects have adapted several physiological mechanisms to regulate their water loss, such as reduced cuticular permeability (CP) and respiratory water loss and increased body water content (Gibbs et al., 1997; Danks, 2000; Bazinet et al., 2010; Benoit and Denlinger, 2010). Consequently, varying water loss rates are reported among insects living in habitats with different moisture levels (Hadley, 1994; Benoit and Denlinger, 2010). Insects also use behavioral adaptations to overcome the risk

of desiccation, such as living in suitable microhabitats (Walters and Mackay, 2003; Benoit et al., 2007), foraging in protected sites (Hu et al., 2012), and making frequent trips to moisture sources (Willmer, 1986).

When more than one species of parasitoid wasps exploit common host resources at the same time in the same habitat, their coexistence can be facilitated by spatial niche partitioning (Amarasekare, 2003; Mills, 2006). Spatial niche difference can be mediated when differences in the intrinsic biological attributes between competing species lead to differences in their abilities to exploit host of varying spatial distribution, such as differences in competitive and dispersal abilities (Lei and Hanski, 1998), ovipositor length (Hanks et al., 2001) and host detection behavior (van Dijken and van Alphen, 1998). In addition, extrinsic environmental factors (temperature, humidity, nutrients, etc.) also vary across a heterogeneous host habitat and thus differently affect competitive abilities of each competing species across space (Amarasekare, 2003; Sorribas et al., 2010).

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Humidity has been found to affect the survival of parasitoid wasps (McFarland and Hoy, 2001; Eman, 2007), and it may be an external mortality factor that selects for a reproductive trait that concentrates on egg production towards early adult life in parasitoid wasps (Jervis et al., 2003; Bernstein and Jervis, 2008). The reproductive success of parasitoid wasps relies not only on their ability to maintain a balanced body water content when foraging for hosts but also on their ability to withstand desiccation during their search for floral food resources, which often are spatially separated from host habitats (Bernstein and Jervis, 2008). However, little is known about the water balance profile and its relationship to relative humidity (RH) preference and survivorship in parasitoid wasps. Such information is critical for predicting the habitat range of these insects and understanding the role of desiccation on shaping the ecological niche of competing parasitoid species.

Evania appendigaster (L.) (Hymenoptera: Evaniidae) and *Aprostocetus hagenowii* (Ratzeburg) (Hymenoptera: Eulophidae) are, respectively, solitary and gregarious egg parasitoids of the American cockroach, *Periplaneta americana* (L.) (Dictyoptera: Blattidae), which is one of the major cosmopolitan pest cockroaches of economic and medical importance (Cornwell, 1968; Lee, 1997, 2007). Females lay their eggs inside oothecae in which 16 cockroach eggs are enclosed in two rows. The immature stages of both parasitoid wasps consume all of the cockroach eggs and take 32–46 days (25–30 °C) to develop before they chew an exit hole on the surface of the ootheca to emerge as adult wasps (Bressan-Nascimento et al., 2010; Cárcamo et al., 2013). *A. hagenowii* is smaller in body size (1.3–2.1 mm in length) but a superior larval competitor compared to *E. appendigaster* (6.5–8.0 mm) (Narasimham, 1984; Kumarasinghe and Edirisinghe, 1987). Both parasitoid species are widespread and commonly found coexisting in areas where *P. americana* occurs (Roth and Willis, 1960; Piper et al., 1978; Narasimham and Sankaran, 1979; Kumarasinghe and Edirisinghe, 1991). At inter- and intraspecific levels, body size has been found to affect water balance in insects because smaller body size means greater body surface area per volume available for cuticular water loss (Hood and Tschinkel, 1990; Addo-Bediako et al., 2001; Kaersgaard et al., 2004; Schilman et al., 2007). Therefore, it is likely that competing parasitoid wasps that differ in body size would experience different levels of desiccation stress in the same habitat and would employ different physiological and behavioral adaptations that lead to their ecological niche difference. In this study, we hypothesized that (1) smaller-sized *A. hagenowii* would experience a higher water loss rate and prefer to live in a more humid area (behavioral adaptation) compared to *E. appendigaster*. Alternatively, physiological adaptations must occur in *A. hagenowii* in order to counteract desiccation risk associated with smaller body size; and (2) *E. appendigaster*, as an inferior larval competitor, must avoid areas dominated by *A. hagenowii*.

We first examined the water balance profile (CP, TBW content, body surface area to volume ratio, and water loss rate) of *E. appendigaster* and *A. hagenowii*. The survivorship of these parasitoids was assessed by rearing them under different RHs in the presence and absence of a food source (sugar solution). Using linearly connected chambers with a humidity gradient, we also investigated the RH preference of both parasitoid species.

2. Materials and methods

2.1. Rearing of *E. appendigaster* and *A. hagenowii*

The colonies of *E. appendigaster* and *A. hagenowii* were obtained from cultures established in the Urban Entomology Laboratory, Vector Control Research Unit, Universiti Sains Malaysia, and maintained using *P. americana* oothecae according to the procedures

described by Tee and Lee (2013). We used unmated parasitoids in the experiments investigating water balance and survivorship under different RH conditions because transfer of sperm and seminal fluids may affect the gravimetrically determined water balance profile and longevity (see review by Arnqvist and Nilsson, 2000). In the RH preference experiment, we used mated parasitoid wasps because (1) *A. hagenowii* is a gregarious species that mates soon after emergence (Edmunds, 1955) and, in other gregarious parasitoids, most females mate before dispersal from their natal patch (Martel and Boivin, 2004) and (2) *E. appendigaster* females that had mated showed greater parasitism activities and fecundity than virgin females (Fox and Bressan-Nascimento, 2006). These mated parasitoids are likely to be engaged more in foraging activities than those unmated individuals.

To acquire enough *E. appendigaster* for the experiments, oothecae aged ≤ 1 week old were exposed individually to a mated female reared inside a round-shaped container (100 ml) supplemented with 10% sugar solution in a cotton-stoppered 2 ml microcentrifuge tube. After 24 h, these exposed oothecae were transferred individually into a 2 ml microcentrifuge tube with a perforated lid (for ventilation) and kept in the rearing condition of 26.4 ± 0.2 °C and $63.2 \pm 0.6\%$ RH. Emergence of parasitoids was checked daily. Newly emerged parasitoids from these parasitized oothecae were sexed and kept separated as unmated wasps for use in the water balance and survivorship experiments. To obtain mated wasps, females were paired with a male in a polyethylene container (100 ml) and observed to ensure that mating occurred before their use in the RH preference experiment. These *E. appendigaster* were 1 days old and oviposition-inexperienced.

Tee and Lee (2013) reported that the numbers of *A. hagenowii* males obtained from a mixed-sex clutch produced by a mated female were limited (average of 5.5 males and 77.4 females). Therefore, to provide sufficient numbers of wasps for use in the experiment, males were obtained from all-male clutches produced from eggs oviposited in oothecae by unmated females. Males and females were obtained by individually exposing ≤ 1 week old oothecae to an unmated and mated female, respectively, inside a 2 ml microcentrifuge tube with a perforated lid. After 24 h, females were removed from the tubes and the exposed oothecae were kept in conditions similar to those for rearing *E. appendigaster*. Newly emerged males from all-male clutches were used as unmated males in the water balance and survivorship experiments. To obtain unmated females, at 1 days before their expected emergence at 36 days post parasitism (Tee and Lee, 2013), mated female-parasitized oothecae were individually placed inside a round-shaped polyethylene container (10 cm height \times 9 cm diameter). The inner wall of the container was smeared with a layer of fluon (Asahi Glass Company, Tokyo, Japan) to prevent the parasitoids from crawling up the wall. Subsequently, we used forceps to make a small hole on the ootheca shell and to clip away males that were emerging from the hole. This procedure was done to prevent females from mating upon their natural emergence, as males actively seek females for mating and each mating lasts for only a few seconds (Tee and Lee, unpublished data). Females that emerged were retained inside the container by a screen lid, and they were used as unmated females in the water balance and survivorship experiments. Mated males and females of *A. hagenowii* were obtained by mixing an all-male clutch into a clutch of unmated females inside the round-shaped container. After 2 h, these wasps were assumed to have mated and were separated by sex for use in the RH preference study.

2.2. Water balance profile

The percentage of total body water (%TBW), CP, and %TBW loss rate of *E. appendigaster* and *A. hagenowii* were determined

gravimetrically. One male or one female of *E. appendigaster* or a group of 50 males or 30 females of *A. hagenowii* were transferred into a glass vial (10 ml) covered with a piece of chiffon cloth to prevent escape of wasps. A total of 15 and 10 vials (replicates) were tested for each sex of *E. appendigaster* and *A. hagenowii*, respectively. The initial masses of these specimens were obtained to the nearest 0.01 mg using a digital analytical balance (Sartorius Extended ED2245, Sartorius AG, Germany). The vials containing the parasitoids were then transferred to a desiccating chamber (11 l). The chamber was held at 24 ± 0.1 °C and maintained at 0–2% RH using 1 kg of anhydrous CaSO_4 (dried at 110 °C for 24 h), which led to a saturation deficit of 21.93 mm Hg inside the chamber. The temperature and RH inside the desiccating chamber were measured using a thermo-hygrometer (Extech Instruments RH520, Waltham, MA, USA). We determined the mass and survival of the parasitoids at 2, 4, 6, 8, 10, 12, and 24 h. After weighing the samples at 24 h, they were dried in an oven at 60 °C for 72 h and then dry masses were obtained. We defined mass loss between each weighing to be the result of water loss. The %TBW was calculated as the difference between initial mass and dry mass divided by initial mass and then multiplied by 100. CP values of parasitoids were calculated as (initial mass – mass at 2 h) (μg)/surface area (cm^2)/time (h)/saturation deficit (mm Hg). Mass (water) loss in the first 2 h was used to calculate CP because maximal water loss occurred during this period as a result of the maximum water gradient between the insect and the desiccating chamber. The effect of reduced water loss rate at subsequent observation periods (due to changing body shape and hemolymph osmolarity and compartmentalization of water in the cuticle and body tissues) on interpretation of CP can be minimized (Appel et al., 1983; Mazer and Appel, 2001). We used Meeh's formula (Meeh, 1879), $S = 12M^{2/3}$, to estimate body surface area of the parasitoid, where S = body surface area (cm^2) and M = initial mass (g) (Mazer and Appel, 2001). There was no significant correlation between CP and initial mass for each sex of each parasitoid wasp, indicating that the use of Meeh's formula provided unbiased estimation of the body surface area and CP values of these parasitoids. The %TBW loss rate was derived from the slope of the linear regression $y = a + bx$ between cumulative %TBW loss and desiccation time (h), where y = cumulative %TBW loss, x = desiccation time (h), a = intercept at y -axis, and b = slope (%TBW loss h^{-1}) (Appel and Tanley, 1999).

2.3. Survival of parasitoids at different RHs in the presence and absence of a sugar solution

We used four saturated salt solutions to manipulate RH within rearing containers to test the effect of RH on survival of parasitoids in the presence and absence of a sugar solution. Based on Winston and Bates (1960), four saturated salt solutions, MgCl_2 , NaBr, NaCl, and KNO_3 , which were reported to produce 33%, 56–59%, 76%, and 91–94% RH, respectively, in a closed environment at 20–30 °C were selected for this experiment. One *E. appendigaster* or a group of 10 *A. hagenowii* were introduced into a 100 ml polyethylene cup covered with a screened lid and then transferred into an air-tight polyethylene container ($37.0 \times 30.0 \times 15.0$ cm) containing a 500 ml beaker filled with one of the four salt solutions. For parasitoids provided with a sugar source, a 10% sugar solution in a cotton-stoppered 2 ml microcentrifuge tube was placed into the 100 ml polyethylene cup. These containers were then kept in environments similar to that used for rearing parasitoids. For each combination of the four RHs and food conditions (with and without sugar solution), a total of 13–18 and 30–40 parasitoids were tested for each sex of *E. appendigaster* and *A. hagenowii*, respectively. The actual RH inside each chamber was measured using a digital thermo-hygrometer during the experiment. The actual RH (\pm SD) within containers held with a saturated salt solution of MgCl_2 ,

NaBr, NaCl, and KNO_3 were $38 \pm 1\%$, $56 \pm 1\%$, $76 \pm 1\%$, and $87 \pm 1\%$ RH, respectively. Rearing containers of parasitoids were named according to their actual RH. Survival of parasitoids was checked every 24 h for *E. appendigaster* kept with and without the sugar solution and *A. hagenowii* with the sugar solution and every 8 h for *A. hagenowii* kept without the sugar solution.

2.4. RH preference

Linearly connected chambers with a RH gradient designed by Walters and Mackay (2003), Ellis et al. (2008) were slightly modified and used in this experiment to investigate the RH preference of parasitoids. The test arena consisted of four transparent polyethylene containers (1000 ml) (Fig. 1). A second smaller polyethylene container (250 ml) was fitted into the top of each 1000 ml container to serve as the activity zone for parasitoids. The bottom portion of the 1000 ml container contained the saturated salt solution. The base of upper container was removed and fitted with a chiffon cloth screen to allow diffusion of water vapor produced by the salt solution within the test chamber and to prevent parasitoids from contacting the salt solution. The four containers were linearly connected using Tygon tubing (1 cm diameter, 2.5 cm length) to allow parasitoids to move from one container to another. Four saturated salt solutions similar to those used for the parasitoid survival experiment were added separately to the bottom portion of the chambers to create a gradient of increasing RH. After being filled with salt solutions, the chambers were left to equilibrate for 24 h before the experiment was started. Because vapor and gas may exchange between these connected chambers, the actual RH in each of the chamber was measured every 15 min for 8 h using a digital thermo-hygrometer. The actual RH (\pm SD) values recorded were $44 \pm 1\%$, $56 \pm 1\%$, $76 \pm 1\%$, and $87 \pm 2\%$ RH. These actual RH values were used to name the respective chambers.

For each RH preference experiment, a total of 20 and 40 individuals of each sex of *E. appendigaster* and *A. hagenowii*, respectively, were used. After the 24 h equilibrium period, 5 *E. appendigaster* and 10 *A. hagenowii* were placed into each of the four RH chambers. Because *A. hagenowii* are positively phototactic (Narasimham, 1984), RH chambers were covered with a red transparent film to minimize the effect of light on the selection of RH chamber by parasitoids. The parasitoids were allowed to acclimatize in the chambers for 2 h. This acclimatization also allowed the humidity within the chambers to stabilize after they were opened for placement of parasitoids (Ellis et al., 2008). Following the 2 h acclimatization period, numbers of parasitoids in each chamber were recorded at 1, 2, 4, 6, and 8 h, and these values were then converted into percentage distribution (relative to total number of parasitoids). A total of 8 and 10 preference tests were conducted for each sex of *E. appendigaster* and *A. hagenowii*, respectively. Placement of the

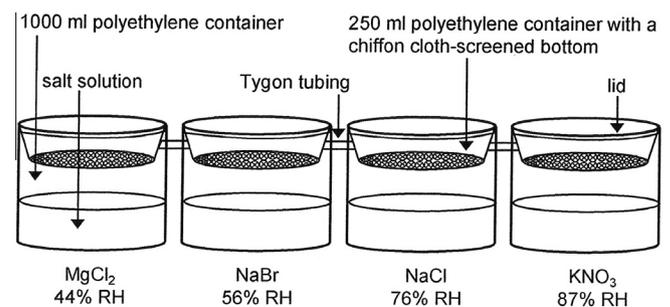


Fig. 1. Diagram of the linearly connected chambers. Four different salt solutions (MgCl_2 , NaBr, NaCl, and KNO_3) were used to create a gradient of increasing RH among the chambers.

linearly connected chambers was also alternated from left to right and right to left to minimize the possible effect of direction on parasitoid RH preference.

2.5. Data analysis

Fresh mass and CP were analyzed and compared between sexes and species using Student's *t*-test. For physiological data measured in ratios with body size as the denominator, the influence of body size may confound the statistical analysis (Packard and Boardman, 1999). To remove this body size effect, we used analysis of covariance (ANCOVA) with fresh mass as the covariate to analyze %TBW and surface area to volume ratio, followed by comparison of means using least significant difference (LSD), to test for differences between sexes and species (Packard and Boardman, 1999). Before analysis, %TBW data were arcsine square-root-transformed to normalize their distribution (Conover and Iman, 1981). For each sex and species of parasitoid, slopes of the regression lines between cumulative %TBW loss and desiccation time (h) were considered to be significantly different if their 95% confidence intervals did not overlap. For each sex and species of parasitoid, the relationship between surface area to volume ratio and %TBW loss rate was examined using Pearson product-moment correlation. The survival data were pooled for each parasitoid sex and species and subjected to probit analysis to estimate their ST_{50} values (time (d) at which 50% of the tested individuals had survived) at different RHs. These values were considered to be significantly different if their 95% fiducial limits did not overlap. The RH preference of parasitoids was determined based on relative distribution of individuals across the four RH chambers. Such compositional data lack an independent characteristic to be subjected to statistical analysis because the sum of all variables equals 100% (constant sum constraint, CSC) (Aitchison, 1986). Therefore, raw percentages were centered-log-ratio transformed to remove the effect of the CSC (Kucera and Malmgren, 1998; Pawlowsky-Glahn and Egozcue, 2006). To determine the effect of RH and time on the distribution of parasitoids, these transformed data were subjected to two-way repeated measures analysis of variance (ANOVA), with RH and time as within-subject factors, because all the groups of male and female *E. appendigaster* ($n = 8$) and *A. hagenowii* ($n = 10$) were subjected to similar RH and time treatments (Ho, 2006). When a significant main or interaction effect was detected, we used separated one-way repeated measures ANOVA to analyze the effect of each significant main factor, followed by separation of means using LSD test. When assumption of sphericity was violated in ANOVA, a Huynh-Felt epsilon was used to adjust the degrees of freedom (Ho, 2006). All statistical analyses were carried out using SPSS version

20 (IBM Corp, New York, NY). The level of significance for all statistical analyses was set at $\alpha = 0.05$.

3. Results

3.1. Water balance of *E. appendigaster* and *A. hagenowii*

For both parasitoid species, females had a significantly greater mean fresh mass than males, but there were no differences in mean %TBW between the sexes and species (Table 1). The CP values of *E. appendigaster* were significantly higher than those of *A. hagenowii*. Within each parasitoid species, there was no significant difference in CP value between males and females. *A. hagenowii* had significantly higher mean surface area to volume ratios than *E. appendigaster*. For each parasitoid species, there was no significant difference in the mean surface area to volume ratio between males and females. For both parasitoid species, the cumulative %TBW loss increased linearly over the desiccation time from 0 to 24 h (Fig. 2 and Table 2). *A. hagenowii* males had a %TBW loss rate of $2.54 \pm 0.09 \text{ h}^{-1}$, which was significantly greater than the rates for *A. hagenowii* females ($0.88 \pm 0.08 \text{ h}^{-1}$) and *E. appendigaster* males ($0.86 \pm 0.09 \text{ h}^{-1}$) and females ($0.97 \pm 0.05 \text{ h}^{-1}$) (Table 2). There were no significant differences in %TBW loss rates among *A. hagenowii* females and *E. appendigaster* males and females. After 24 h of desiccation, the mortality of *A. hagenowii* males and females and *E. appendigaster* males and females were $63.1 \pm 7.3\%$, $26.8 \pm 2.3\%$, $25.1 \pm 1.4\%$, and $26.5 \pm 1.6\%$, respectively. There was

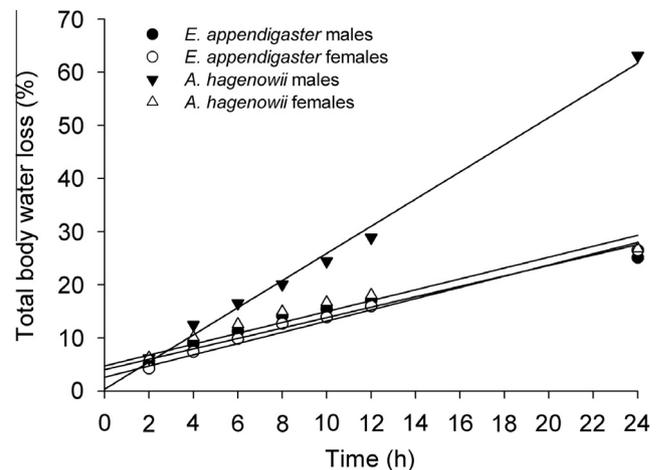


Fig. 2. Cumulative% total body water loss of males and females of *E. appendigaster* and *A. hagenowii* with desiccation time (0–24 h) at $24.0 \pm 0.1 \text{ }^\circ\text{C}$ and 0–2% RH.

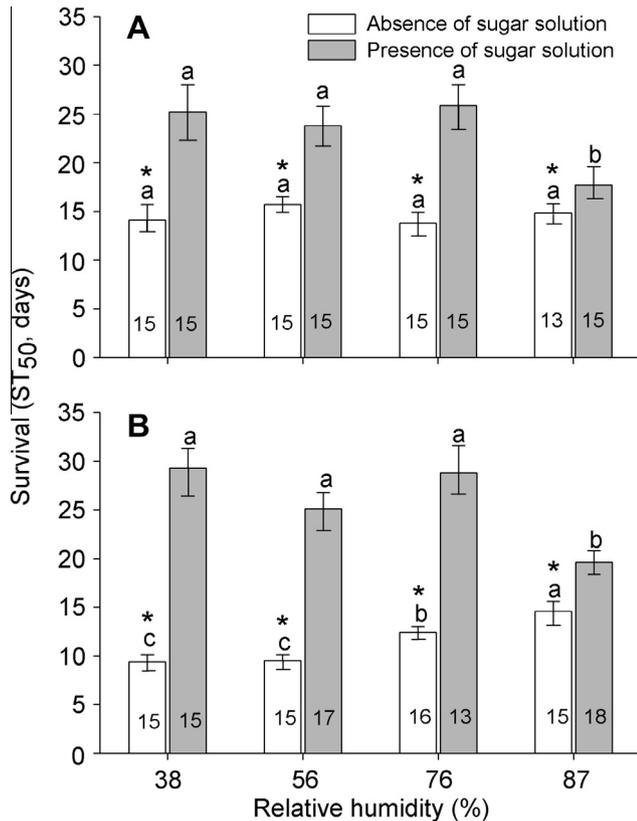
Table 1
Fresh mass, percentage of total body water, cuticular permeability, and surface area to volume ratio (mean \pm SE) of males and females of *E. appendigaster* and *A. hagenowii*.

Physiological parameters	Sex	<i>E. appendigaster</i> ($n = 15$)	<i>A. hagenowii</i> ($n = 10$)	Test statistics
^a Fresh mass (mg)	♂	22.31 \pm 0.46a(a)	0.1864 \pm 0.007a(b)	$t_{14} = -47.80, P < 0.001$ $t_{14} = -39.53, P < 0.001$
	♀	24.75 \pm 0.62b(a)	0.3991 \pm 0.010b(b)	
		$t_{28} = -3.12, P = 0.004$	$t_{18} = -18.23, P < 0.001$	
^b %TBW	♂	58.11 \pm 1.43	68.96 \pm 0.60	$F_{1, 22} = 0.01, P = 0.927$ $F_{1, 22} = 0.35, P = 0.540$
	♀	59.39 \pm 0.60	62.73 \pm 0.67	
		$F_{1, 27} = 6.40, P = 0.432$	$F_{1, 17} = 3.67, P = 0.072$	
^a CP ($\mu\text{g cm}^{-2} \text{ h}^{-1} \text{ mm Hg}^{-1}$)	♂	32.69 \pm 3.22(a)	9.33 \pm 1.78(b)	$t_{21} = -6.35, P < 0.001$ $t_{19} = -6.02, P < 0.001$
	♀	30.38 \pm 2.92(a)	11.08 \pm 1.33(b)	
		$t_{28} = 0.53, P = 0.566$	$t_{18} = -0.78, P = 0.441$	
^b Surface area to volume ratio ($\text{cm}^2 \text{ g}^{-1}$)	♂	42.68 \pm 0.29(a)	210.60 \pm 2.49(b)	$F_{1, 22} = 84.11, P < 0.001$ $F_{1, 22} = 198.23, P < 0.001$
	♀	41.26 \pm 0.35(a)	163.19 \pm 1.38(b)	
		$F_{1, 17} = 0.04, P = 0.848$	$F_{1, 17} = 0.03, P = 0.859$	

Means in the same column followed by different letters and means in the same row followed by different letters in parentheses are significantly different (^aStudent's *t*-test; ^bANCOVA (fresh weight as covariate) followed by LSD test; $P < 0.05$).

Table 2Statistics for the linear regression between cumulative %TBW loss and desiccation time (h) for males and females of *E. appendigaster* and *A. hagenowii*.

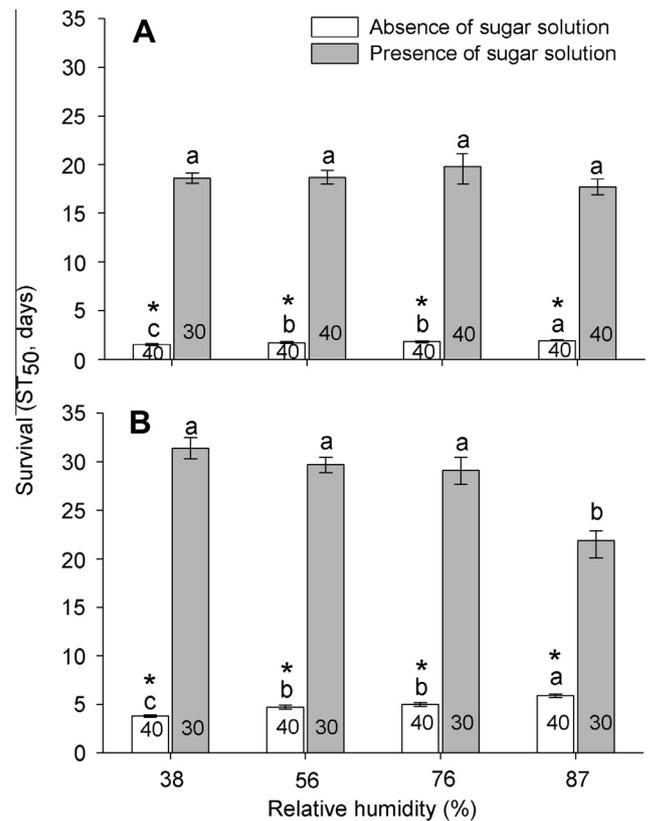
Species	Sex	^a Slope ± SE	Intercept ± SE	F	r ²	P
<i>E. appendigaster</i>	♂	0.86 ± 0.09a	5.76 ± 0.98	102.63	0.954	<0.0001
	♀	0.97 ± 0.05a	3.83 ± 0.55	425.03	0.988	<0.0001
<i>A. hagenowii</i>	♂	2.54 ± 0.09b	0.50 ± 1.09	731.25	0.993	<0.0001
	♀	0.83 ± 0.06a	6.38 ± 0.64	229.50	0.979	<0.0001

^a Means followed by different letters are significantly different based on non-overlapping of their 95% confidence intervals.**Fig. 3.** Survival (ST₅₀, d) of *E. appendigaster* males (A) and females (B) when kept at 38%, 56%, 76%, and 87% RH in the absence and presence of food (10% sugar solution). Different letters and asterisks above bars denote significant differences in survival of parasitoids between different RHs in each food condition and between different food conditions in each RH, respectively. The vertical line on each bar represents the ±95% fiducial limit. Numbers inside bars indicate sample size.

no correlation between surface area to volume ratio and %TBW loss rate for males and females of *E. appendigaster* (Pearson's correlation coefficient, males, $r = -0.190$, $n = 15$, $P = 0.497$; females, $r = 0.426$, $n = 15$, $P = 0.113$). For *A. hagenowii*, surface area to volume ratio was positively correlated with %TBW loss rate in males (Pearson's correlation coefficient, $r = 0.944$, $n = 10$, $P < 0.001$) and females ($r = 0.739$, $n = 10$, $P = 0.015$).

3.2. Survival time of *E. appendigaster* reared at different RHs

In the absence of food, the survival times of *E. appendigaster* males maintained at different RHs were not significantly different, with ST₅₀ values ranging from 13.8 to 15.7 days (Fig. 3). When food was not present, females survived significantly longer at 87% RH (14.6 days) and significantly shorter at 38% and 56% RH (9.4–9.5 days). When food was present, there were no significant differences in the ST₅₀ of males (23.8–25.9 days) and females (25.1–

**Fig. 4.** Survival (ST₅₀, d) of *A. hagenowii* males (A) and females (B) when kept at 38%, 56%, 76%, and 87% RH in the absence and presence of food (10% sugar solution). Different letters and asterisks above bars denote significant differences in survival of parasitoids between different RHs in each food condition and between different food conditions in each RH, respectively. The vertical line on each bar represents the ±95% fiducial limit. Numbers inside bars indicate sample size.

29.3 days) reared at 38–76%. In comparison, males and females with food present lived for a significantly shorter amount of time at 87% RH.

3.3. Survival time of *A. hagenowii* reared at different RHs

In the absence of food, the ST₅₀ of males was 1.9 days at 87% RH, which was significantly higher than those of males maintained at the other RHs (Fig. 4). The ST₅₀ of males without food was significantly shorter at 38% RH (1.5 days) compared to males reared at the other RHs. When food was not present, the ST₅₀ values of females were 5.9 and 3.8 days at 87% and 38% RH, respectively, which were the highest and lowest values among the RHs evaluated. When food was present, there was no significant difference among the ST₅₀ values of males (17.7–19.8 days) at all RH values. There was no significant difference in the ST₅₀ values of females at 38%, 56%, and 76% RH (29.1–31.4 days), but these values were significantly greater than the 21.9 days recorded at 87% RH.

3.4. RH preference of *E. appendigaster*

The distribution of males and females within the RH chambers were significantly affected by RH (males: $F_{1.5, 10.3} = 30.25$, $P < 0.001$; females: $F_{3, 21} = 30.25$, $P < 0.001$; Fig. 5) but not by time and RH \times time interaction effect (male: time, $F_{4, 28} = 1.07$, $P = 0.391$; RH \times time, $F_{6.7, 46.8} = 1.16$, $P = 0.344$; females: time, $F_{3.1, 21.9} = 1.30$, $P = 0.301$; time \times RH, $F_{12, 84} = 1.37$, $P = 0.196$). At each time interval, except 4-h time point, the percentage of males in the 76% RH was significantly higher than that in the 44% RH (1 h, $F_{1.3, 8.9} = 8.52$, $P = 0.014$; 2 h, $F_{3, 21} = 8.26$, $P = 0.001$; 4 h, $F_{3, 21} = 1.73$, $P = 0.193$; 6 h, $F_{2.9, 20} = 13.64$, $P < 0.001$; 8 h, $F_{1.5, 10.3} = 6.77$, $P = 0.018$). Because there were no significant effects of time and RH \times time interaction, data were pooled from all time intervals to interpret the overall influence of RH on the distribution of males. Analysis of the pooled data showed that males were significantly more frequent in the 76% RH chamber ($32.4 \pm 1.6\%$) and least frequent in the 44% RH chamber ($18.0 \pm 0.8\%$) ($F_{2.3, 90.1} = 25.15$, $P < 0.001$). The percentages of males residing in the 56% and 87% RH chambers (24.1 – 25.5%) were significantly lower than that in 76% RH chamber but these percentages were significantly higher than that in 44% RH chamber. At each time interval, the percentage of females in the 87% RH chamber was significantly higher than that in 44% RH chamber (1 h, $F_{1.3, 8.9} = 8.52$, $P = 0.014$; 2 h, $F_{3, 21} = 8.26$, $P = 0.001$; 4 h, $F_{3, 21} = 1.73$, $P = 0.193$; 6 h, $F_{2.9, 20} = 13.64$, $P < 0.001$; 8 h, $F_{1.5, 10.3} = 6.77$, $P = 0.018$). When data were pooled from all time intervals for each RH (due to no significant effects of time and RH \times time interaction), we found that the percentage of females in 87% RH chamber ($29.9 \pm 1.1\%$) was significantly higher than those of the other RH chambers (20.5 – 24.9%) ($F_{2.6, 102.4} = 20.13$, $P < 0.001$).

3.5. RH preference of *A. hagenowii*

RH and RH \times time interaction effect had a significant influence on the distribution of both males (RH, $F_{3, 27} = 21.62$, $P < 0.001$; time, $F_{4, 36} = 1.51$, $P = 0.221$; RH \times time, $F_{7.1, 63.42} = 10.31$, $P < 0.001$; Fig. 6) and females (RH, $F_{3, 27} = 10.75$, $P < 0.001$; time, $F_{4, 36} = 0.27$, $P = 0.899$; RH \times time, $F_{7.7, 69.3} = 5.05$, $P < 0.001$) within the RH chambers. At 2-h time interval, the percentages of males

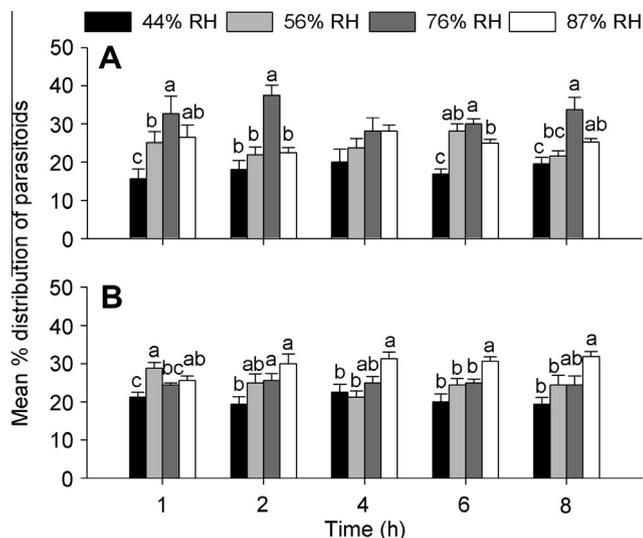


Fig. 5. Mean% distribution of *E. appendigaster* males (A) and females (B) in linearly connected chambers with a gradient of 44%, 56%, 76%, and 87% RH. Different letters above each bar indicate significant differences in the distribution of parasitoids among RH chambers at each time interval (LSD test, $P < 0.05$). The vertical line on each bar represents standard error of the mean ($n = 8$).

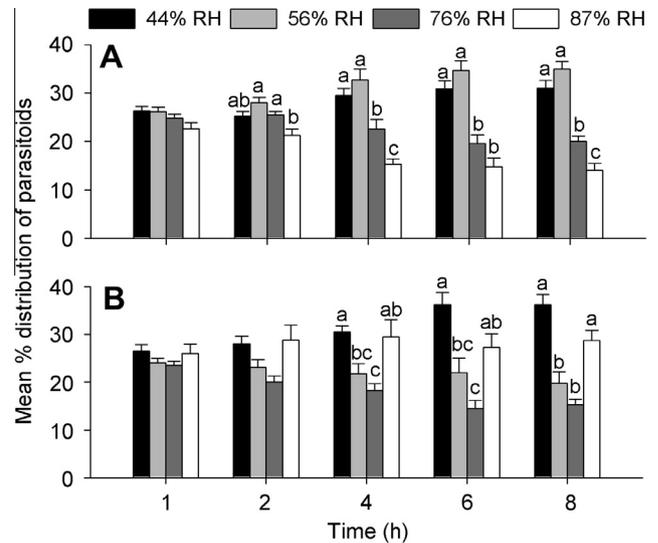


Fig. 6. Mean% distribution of *A. hagenowii* males (A) and females (B) in linearly connected chambers with a gradient of 44%, 56%, 76%, and 87% RH. Different letters above each bar indicate significant differences in the distribution of parasitoids among RH chambers at each time interval (LSD test, $P < 0.05$). The vertical line on each bar represents standard error of the mean ($n = 10$).

in 56% and 76% RH chambers were significantly greater than that in 87% RH chamber ($F_{3, 27} = 5.54$, $P = 0.004$), whereas percentage of males in 44% RH chamber did not differ significantly from those of the other RH chambers. However, at between 4-h and 8-h time interval, males showed a preference for the drier end of the RH gradient, as 29.5–35.0% of parasitoids were distributed in the 44% and 56% RH chambers; 19.6–22.5% of the wasps were found in the 76% RH chamber and 14.0–15.3% were present in the 87% RH chamber (4 h, $F_{3, 27} = 13.25$, $P < 0.001$; 6 h, $F_{2.7, 24.2} = 17.76$, $P < 0.001$; 8 h, $F_{1.8, 16.6} = 30.77$, $P < 0.001$). There were no significant differences in the percentages of females in different RH chambers at 1-h and 2-h time intervals (1 h, $F_{3, 27} = 0.66$, $P = 0.581$; 2 h, $F_{3, 27} = 2.07$, $P = 0.127$). At 4-h and 6-h time intervals, females were most frequent in both the driest (30.5–36.3% at 44% RH) and wettest (27.3–29.5% at 87% RH) ends of the humidity gradient, and these percentages did not differ significantly from each other (4 h, $F_{1.9, 16.6} = 5.97$, $P = 0.013$; 6 h, $F_{3, 27} = 10.39$, $P < 0.001$). These percentages for the 44% RH chamber were significantly greater than those documented for the chambers with intermediate RH levels (21.8–22.0% in 56% RH and 14.5–18.3% in 76% RH). At 8-h time interval, the percentages of females in 44% and 87% RH chambers (27.3–36.3%) were significantly higher than those in the 56% and 76% RH chambers (14.5–22.0%) ($F_{1.8, 16.6} = 30.77$, $P < 0.001$).

4. Discussion

In this study, the smaller-sized *A. hagenowii* females had a water loss rate that was similar to that of bigger-sized *E. appendigaster* males and females. The low CP found in *A. hagenowii* females might have contributed to their lower water loss rate. However, as revealed by the positive correlation between surface area to volume ratio and water loss rate, the low CP in *A. hagenowii* males did not result in low water loss rate due to their high surface area to volume ratio. These differences in the water balance profile may reflect differences in life history traits and mating strategies between these two oothecal parasitoids. In *A. hagenowii*, we found a positive correlation between surface area to volume ratio and water loss rate and a great difference in size between genders, with fresh mass of females two times greater than that of males. This

difference is expected to be even greater for males that originate from mix-sexed broods due to greater resource competition from females of larger size. Both sexes of *A. hagenowii* wasps undergo multiple mating events, which occur on site soon after emergence (Edmunds, 1955; Tee and Lee, unpublished data). Therefore, by developing as a gregarious parasitoid, it is possible that most of the resources within a host can be optimally allocated to females for greater fitness return (increased longevity, fecundity, and desiccation resistance), with a smaller proportion invested in males just for mating. In contrast, *E. appendigaster* females are monandrous and males are polygynous (Yeh and Mu, 1994). Both sexes engage in desiccation risk-associated activities such as host searching in females and mate finding in males. Therefore, it is critical for both males and females to possess the low water loss trait. Moreover, because they are solitary parasitoids, all resources within a host can be fully utilized for such activities.

Having high body water content is one strategy for insects to be less susceptible to desiccation (Danks, 2000). However, we found that the %TBWs of *E. appendigaster* males and females (58.11–59.39%) and *A. hagenowii* females (62.73%) were below the average value of 70% for most insects (Hadley, 1994). This suggests that these oothecal parasitoids may have shifted from conserving more water to having high lipid reserves, as low %TBW is commonly associated with high lipid reserves in insects (Benoit and Denlinger, 2010). Lipids are important general reserves in adult parasitoid wasps because, unlike most other insects, most parasitoid wasps lack the ability to synthesize lipids from their carbohydrate-rich foods (such as nectar and extrafloral nectar) and can only obtain a small amount of lipids through feeding on host hemolymph (Giron and Casas, 2003; Giron et al., 2004; Visser and Ellers, 2008; Visser et al., 2010). Therefore, the lipid reserves in adult parasitoid wasps are acquired and accumulated during larval development (Jervis et al., 2008). *E. appendigaster* and *A. hagenowii* are synovigenic parasitoids, which means that they emerge with some eggs in their ovarioles and that eggs continue to mature during their adult life (Narasimham, 1984; Yeh and Mu, 1994; Zhang et al., 2010). For parasitoids, lipid reserves can be used to produce eggs and, when body sugar and stored glycogen are low, they can also be mobilized as an energy source to fuel body maintenance and flight activity (Ellers and van Alphen, 1997; Ellers et al., 1998; Rivero and Casas, 1999; Giron et al., 2002; Strand and Casas, 2008). Thus, the amount of lipid reserves plays a crucial role in parasitoid fitness, as it has been shown to be positively correlated with fecundity, longevity, and dispersal distance (Ellers et al., 1998; Rivero and West, 2002). In contrast to the species studied herein, Yoder and Hoy (1998) reported that *Ageniaspis citricola* (Logvinovskaya), an egg and larval parasitoid of the citrus leafminer *Phyllocnistis citrella* (Stainton), had a high %TBW, which might be an important way for this parasitoid to reduce desiccation risk, as its water loss rate ranged from 3.07 to 4.86% h⁻¹.

In our humidity preference study, both *E. appendigaster* and *A. hagenowii* females preferred the most humid chamber (87% RH). In the absence of the sugar solution, the survival times of *E. appendigaster* and *A. hagenowii* females in this chamber (14.6 and 5.9 days) were 1.5 times greater than those in the 38% RH chamber (9.4 and 3.8 days, respectively). This increased survival time may explain females' preference for a more humid area. However, residing in a humid area as a behavioral adaptation to reduce desiccation risk costs them current (host searching) and future (food searching) reproductive opportunities. Water loss rates (%TBW loss h⁻¹) of *A. hagenowii* and *E. appendigaster* (0.83 and 0.97% h⁻¹, respectively) females are among the lowest of mesic species (based on the classification of insects using water loss rates (% h⁻¹) introduced by Hadley (1994), where xeric ≤ 0.8% h⁻¹; mesic = 0.8–2.0% h⁻¹; hygic ≥ 2.0% h⁻¹). This finding suggests that desiccation risk may not be a primary factor in humidity

preference of these parasitoids in mesic and hygic environments. Alternatively, preference for a humid area may indicate the use of humidity as a host habitat location cue for oothecal parasitoids to locate cockroach harborage sites. Due to high water loss rates (Appel et al., 1983), their synanthropic cockroach hosts (*P. americana*, *Periplaneta fuliginosa* (Serville), and *Blatta orientalis* (L.)) often distribute patchily in humid microhabitats around residential properties, such as tree holes, woodpiles, sewers, and crawlspaces (Fleet et al., 1978; Appel and Rust, 1985; Thoms and Robinson, 1987; Brenner, 1988; Tee et al., 2011a). The preference of *E. appendigaster* males for a humid area (76% RH) also supports the use of a humidity gradient as a host location cue for *E. appendigaster* females. Because mating opportunity is not immediately available upon emergence of solitary parasitoids (Martel and Boivin, 2004), preference for a humid area by evaniid males might be a strategy for them to access females, which also are attracted to humid habitats. The possibility of a humidity gradient as a cue for habitat location has also been proposed for *Lariophagus distinguendus* (Forster), a larval and pupal parasitoid wasp of beetle pests of stored products, and *Brachymeria* spp., pupal parasitoid wasps of the gypsy moth; both parasitoids were attracted to low humidity areas which, respectively, corresponded to the habitats of beetle hosts in grain stores (Steidle and Reinhard, 2003) and gypsy moth pupae found in defoliated areas at the top of the canopy and along the margin of forest openings (Minot and Leonard, 1976; Weseloh, 1979).

On the other hand, attraction of *A. hagenowii* females to a drier area may be an adaptive behavior that facilitates leaving the humid host habitat and dispersing towards a relatively drier surrounding environment to search for floral and extrafloral nectar. In the absence of the sugar solution, *A. hagenowii* females were short lived (3.8–5.9 days in this study), and most of their eggs matured 1 days after emergence (Narasimham, 1984; Zhang et al., 2010). Thus, the time window for *A. hagenowii* females to find cockroach hosts that are patchily distributed would be limited in the absence of food sources. The risk of time limitation can be reduced by gaining access to food sources, as our results demonstrated: Longevity of *A. hagenowii* females provided with 10% sugar solution was 5.8–8.3 times greater than that of wasps without sugar solution in 38–76% RH chambers. A significantly lower increment (3.7 times) was recorded in the 87% RH chamber provided with sugar solution, probably due to the growth of fungus on the cotton containing the sugar solution, which may have limited access to food or resulted in a fungal infection among the wasps. Feeding on floral food resources is common among hymenopteran parasitoids (Jervis et al., 1993) and has been shown to increase the fitness of parasitoids in agricultural sites (Tyljanakis et al., 2004; Lee and Heimpel, 2008; Rusch et al., 2013). Similarly, in an urban landscape, abundances of parasitoids were positively correlated with the diversity of flowers around residential and commercial buildings (Bennett and Gratton, 2012). Narasimham (1984) showed that *A. hagenowii* provided with mucilage of *Hibiscus rosa-sinensis* L. and nectar of *Leucas aspera* (Willd.) had slightly greater longevity compared to wasps without food sources.

In this study, *A. hagenowii* males were also attracted to the drier chamber (44% RH). This similarity in humidity preference between sexes suggests that males may utilize the same humidity cue used by females for food searching to access both female mates and food sources at feeding sites. Although gregarious parasitoids mate on site upon emergence, it is an advantage for males to disperse after on site mating to seek more female mates elsewhere (Martel and Boivin, 2004). Occurrence of mating at feeding sites has not been documented in parasitoid wasps, but it may be one of their mating strategies, as male and female parasitoid wasps have been found visiting flowers for floral food resources (Jervis et al., 1993; Godfray and Cook, 1997).

In contrast, the driest humidity chamber (44% RH) was the least preferred by *E. appendigaster*. *E. appendigaster* (males, 13.8–15.7 days; females, 9.4–14.6 days) lived longer than *A. hagenowii* (males, 1.5–1.9 days; females, 3.8–5.9 days) in the absence of sugar solution in the 38–87% RH chambers. This suggests that *E. appendigaster* is less susceptible to time limitation than *A. hagenowii*. Moreover, *E. appendigaster* is an inferior larval competitor compared to *A. hagenowii*, as *A. hagenowii* can successfully develop in *E. appendigaster*-parasitized oothecae (Kumarasinghe and Edirisinghe, 1987). In a survey of oothecal parasitoids from residential areas in Texas and Louisiana, 96.5% of the 113 parasitized *P. americana* oothecae collected from the outer surface of buildings contained *A. hagenowii*, whereas the remaining 3.5% was attributed to *E. appendigaster* (Piper et al., 1978). *E. appendigaster* has been found to be a predominant species in sewer systems (Tee and Lee, unpublished data) and has been observed to prefer humid areas such as basements, crawl spaces, and culverts (Piper et al., 1978). *A. hagenowii* were not detected in sentinel oothecae placed inside sewer systems heavily infested with *P. americana* (Reierson et al., 2005; Tee et al., 2011b). In this study, similarity in water loss rates, difference in RH preference, and different effects of a sugar food source on longevity were documented for these two oothecal parasitoids. These results suggest that *E. appendigaster*, with its longer life span and better dispersal ability (as a result of larger size), may outcompete *A. hagenowii* in colonizing humid cockroach habitats that are far from or have restricted access to floral food resources.

Previous comparative studies have demonstrated the effect of body size and CP on water loss variation among sympatric insects living in mesic environments. Consequently, differences in water loss resulted in temporal and spatial differences in the exploitation of resources among these insects. For example, larger-sized *Macrotermes carbonarius* (Hagen) (11.3–69.2 mg fresh weight) had lower water loss rates than the smaller-sized *Macrotermes gilvus* (Hagen) (5.1–26.5 mg), even though both congeneric termite species had similar CP values (14.7–51.5) (Hu et al., 2012). *M. carbonarius* had been found to forage efficiently on the ground and in an exposed condition during humid seasons ($\geq 70\%$ atmospheric RH). In contrast, *M. gilvus* were documented to restrict their foraging activities within below-ground tunnels and concealed mud tubes (Hu et al., 2012). In another study, differences in CP values among larvae of two sympatric tortoise beetles, *Charidotella bicolor* (F.) and *Deloyala duttata* (Olivier), had been found to result in differences in their vulnerability to desiccating environments (Hull-Sanders et al., 2003). Although larvae of both tortoise beetle species had similar body sizes, higher CP values in *C. bicolor* larvae (17.3–23.2) contributed to their higher water loss rates than those of *D. duttata* (9.1–11.7). In this study, our results showed that reduced CP is critical for *E. appendigaster* and *A. hagenowii* to counteract the desiccation risk associated with small body sizes; the smaller the body size, the lower the CP values in these oothecal parasitoid wasps. This adaptive physiological trait resulted in lower water loss rates in these ootheca parasitoids (except *A. hagenowii* males) compared to their cockroach hosts of larger body sizes (*P. americana*, *Periplaneta australasiae* (F.) and *B. orientalis*: CP, 43.1–53.7; water loss rate, 1.82–2.44% h⁻¹) (Appel et al., 1983).

Several field studies have been conducted to evaluate the efficacy of *A. hagenowii* in controlling *P. americana*. The effect of release of *A. hagenowii* around tree holes (Suiter et al., 1998), crevices around buildings (Tee et al., 2011b), sewer systems (Reierson et al., 2005), plumbing chases (Pawson and Gold, 1993), basements (Roth and Willis, 1954), and a simulated kitchen (Hagenbuch et al., 1989) has been studied. Among these studies, high parasitism rates (50–100%) of oothecae were documented for releases around tree holes, basements, and a simulated kitchen, whereas other release sites had low to moderate parasitism rates

(0–44%). However, these cockroach habitats differed in terms of RH and food accessibility (availability of or varying distances from floral food resources) and thus may have had different levels of attraction and retention of the wasps. Preference for a dry environment and limited accessibility of floral food resources may explain the low performance of *A. hagenowii* in controlling *P. americana* in confined humid environments. Our results suggest that the use of augmentative releases of *A. hagenowii* in humid man-made structures to control *P. americana* needs to be reevaluated.

5. Conclusion

We found a distinct RH preference between *E. appendigaster* and *A. hagenowii*. With the exception of females of both parasitoids showing an overlapping preference for the most humid chamber, the parasitoids species exhibited contrasting RH preferences: *A. hagenowii* males and females preferred to forage in the driest chamber, whereas *E. appendigaster* males and females preferred a more humid area. This difference in RH preference was not due to differences in water requirements, as *A. hagenowii* females and both sexes of *E. appendigaster* had similar %TBW and water loss rates. The difference in RH preference may relate to fitness gain associated with each parasitoid's life history. For gregarious *A. hagenowii*, there was a positive relationship between surface area to volume ratio and water loss rate in males and females. Females were larger than males, and, thus, had lower surface area to volume ratio. This disproportionate allocation of resources within a host to produce females of larger size together with a low CP enable *A. hagenowii* females to have a low water loss rate, which facilitates their foraging in a dry environment. Because *A. hagenowii* females were short lived in the absence of food sources, attraction to a dry environment serves as an adaptive behavior that assists them in exploiting floral food resources for enhanced survival. In contrast, as an inferior larval competitor, longer-lived solitary *E. appendigaster* would perform better than *A. hagenowii* in colonizing humid areas far from or with limited accessibility to floral food resources. These findings are critical for understanding the behavioral and physiological adaptations that allow *E. appendigaster* and *A. hagenowii* to co-exist and developing efficient ways to use these oothecal parasitoids for biological control of *P. americana*. Because *E. appendigaster* and *A. hagenowii* prefer different RH zones, augmentative release of both parasitoid species may provide better control of *P. americana* that inhabit various microhabitats in urban environments.

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References

- Amarasekare, P., 2003. Competitive coexistence in spatially structured environments: a synthesis. *Ecol. Lett.* 6, 1109–1122.
- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2001. Revisiting water loss in insects: a large scale view. *J. Insect. Physiol.* 47, 1377–1388.
- Aitchison, J., 1986. *The Statistical Analysis of Compositional Data*. Chapman & Hall, London, UK.
- Appel, A.G., Reierson, D.A., Rust, M.K., 1983. Comparative water relations and temperature sensitivity of cockroaches. *Comp. Biochem. Physiol. A* 74, 357–361.
- Appel, A.G., Rust, M.K., 1985. Outdoor activity and distribution of the smokybrown cockroach, *Periplaneta fuliginosa* (Dictyoptera: Blattellidae). *Environ. Entomol.* 14, 669–673.
- Appel, A.G., Tanley, M.J., 1999. Water composition and loss by body color and form mutants of the German cockroach (Dictyoptera: Blattellidae). *Comp. Biochem. Physiol. A* 122, 415–420.

- Arnqvist, G., Nilsson, T., 2000. The evolution of polyandry: multiple mating female fitness in insects. *Anim. Behav.* 60, 145–164.
- Bazinnet, A.L., Marshall, K.E., MacMillan, H.A., Williams, C.M., Sinclair, B.J., 2010. Rapid changes in desiccation resistance in *Drosophila melanogaster* are facilitated by changes in cuticular permeability. *J. Insect. Physiol.* 56, 2006–2012.
- Bennett, A.B., Gratton, C., 2012. Local and landscape scale variables impact parasitoid assemblages across an urbanization gradient. *Landscape Urban Plan.* 104, 26–33.
- Benoit, J.B., Denlinger, D.L., 2010. Meeting the challenges of on-host and off-host water balance in blood feeding arthropods. *J. Insect. Physiol.* 56, 1366–1376.
- Benoit, J.B., Lopez-Martinez, C., Elnitsky, M.A., Lee Jr., R.E., Denlinger, D., 2007. Moist habitats are essential for adults of the Antarctic midge, *Belgica antarctica* (Diptera: Chironomidae), to avoid dehydration. *Eur. J. Entomol.* 104, 9–14.
- Bernstein, C., Jervis, M.A., 2008. Food-searching in parasitoids: the dilemma of choosing between 'immediate' or future fitness gains. In: Wajnberg, E., Bernstein, C., van Alphen, J.J.M. (Eds.), *Behavioural Ecology of Parasitoids: From Theoretical Approaches to Field Applications*. Blackwell Publishing, Oxford, UK, pp. 129–171.
- Brenner, R.J., 1988. Focality and mobility of some peridomestic cockroaches in Florida (Diptera: Blattellidae). *Ann. Entomol. Soc. Am.* 81, 581–592.
- Bressan-Nascimento, S., Fox, E.G.P., Pilizi, L.G.T., 2010. Effects of different temperatures on the life history of *Evania appendigaster* L. (Hymenoptera: Evaniidae), a solitary oothecal parasitoid of *Periplaneta americana* L. (Diptera: Blattellidae). *Biol. Control* 52, 104–109.
- Cárcamo, M.C., Felchicher, F., Duarte, J.P., Krüger, R.F., Vianna, É.E.S., Ribeiro, P.B., 2013. Thermal requirement of *Aprostocetus hagenowii* (Ratzeburg, 1852) (Hymenoptera, Eulophidae) reared in oothecae of *Periplaneta americana* (Linnaeus, 1758) (Blattaria, Blattellidae). *Biotemas* 26, 271–275.
- Conover, W.J., Iman, R.L., 1981. Rank transformations as a bridge between parametric and non-parametric statistics. *Am. Stat.* 35, 124–129.
- Cornwell, P.B., 1968. *The Cockroach*, vol. 1. A Laboratory Insect and an Industrial Pest, Hutchinson, London, UK.
- Danks, H.V., 2000. Dehydration in dormant insects. *J. Insect Physiol.* 46, 837–852.
- Edmunds, L.R., 1955. Biological notes on *Tetrastichus hagenowii* (Ratzeburg), a chalcidoid parasite of cockroach eggs (Hymenoptera: Eulophidae; Orthoptera: Blattellidae). *Ann. Entomol. Soc. Am.* 48, 210–213.
- Ellers, J., van Alphen, J.J.M., 1997. Life history evolution in *Asobara tabida*: plasticity in allocation of fat reserves to survival and reproduction. *J. Evol. Biol.* 10, 771–785.
- Ellers, J., van Alphen, J.J.M., Sevenster, J.G., 1998. A field study of size-fitness relationships in the parasitoid *Asobara tabida*. *J. Anim. Ecol.* 67, 318–324.
- Ellis, M.B., Nicolson, S.W., Crewe, R.M., Diemann, V., 2008. Hygro-preference and brood care in the honey bee (*Apis mellifera*). *J. Insect Physiol.* 54, 1516–1521.
- Emana, G.D., 2007. Comparative studies of the influence of relative humidity and temperature on the longevity and fecundity of the parasitoid, *Costesia flavipes*. *J. Insect Sci.* 7, 19.
- Fleet, R.R., Piper, G.L., Frankie, G.W., 1978. Studies on the population ecology of the smokybrown cockroach, *Periplaneta fuliginosa*, in a Texas outdoor urban environment. *Environ. Entomol.* 7, 807–814.
- Fox, E.G.P., Bressan-Nascimento, S., 2006. Biological characteristics of *Evania appendigaster* (L.) (Hymenoptera: Evaniidae) in different densities of *Periplaneta americana* (L.) oothecae (Blattodea: Blattellidae). *Biol. Control* 36, 183–188.
- Gibbs, A.G., Chippindale, A.K., Rose, M.R., 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *J. Exp. Biol.* 200, 1821–1832.
- Giron, D., Casas, J., 2003. Lipogenesis in an adult parasitic wasp. *J. Insect Physiol.* 49, 141–147.
- Giron, D., Pincebourde, S., Casas, J., 2004. Life-time gain of host-feeding in a synovigenic parasitic wasp. *Physiol. Entomol.* 29, 436–442.
- Giron, D., Rivero, A., Mandon, N., Darrouzet, E., Casas, J., 2002. The physiology of host feeding in parasitic wasps: implications for survival. *Funct. Ecol.* 16, 750–757.
- Godfrey, H.C.J., Cook, J.M., 1997. Mating systems of parasitoid wasps. In: Choe, J.C., Crespi, B.J. (Eds.), *The Evolution of Mating Systems in Insects and Arachnids*. Cambridge University Press, Cambridge, UK, pp. 211–225.
- Hadley, N.F., 1994. *Water Relations of Terrestrial Arthropods*. Academic Press, NY.
- Hagenbuch, B.E., Patterson, R.S., Koehler, P.G., 1989. Biological control of the American cockroach (Orthoptera: Blattellidae) with inundative releases of *Tetrastichus hagenowii* (Hymenoptera: Eulophidae). *J. Econ. Entomol.* 82, 90–94.
- Hanks, L.M., Millar, J.G., Paine, T.D., Wang, Q., Paine, E.O., 2001. Patterns of host utilization by two parasitoids (Hymenoptera: Braconidae) of the eucalyptus longhorned borer (Coleoptera: Cerambycidae). *Biol. Control* 21, 152–159.
- Ho, R., 2006. *Handbook of univariate and multivariate data analysis and interpretation with SPSS*. Chapman & Hall/CRC, Boca Raton, FL.
- Hood, W.G., Tschinkel, W.R., 1990. Desiccation resistance in arboreal and terrestrial ants. *Physiol. Entomol.* 15, 23–35.
- Hull-Sanders, H.M., Appel, A.G., Eubanks, M.D., 2003. Comparative water relations of adult and juvenile tortoise beetles: differences among sympatric species. *Comp. Biochem. Physiol. A* 135, 625–634.
- Hu, J., Neoh, K.B., Appel, A.G., Lee, C.Y., 2012. Subterranean termite open-air foraging and tolerance to desiccation: comparative water relation of two sympatric *Macrotermes* spp. (Blattodea: Termitidae). *Comp. Biochem. Physiol. A* 161, 201–207.
- Jervis, M.A., Ellers, J., Harvey, J.A., 2008. Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. *Annu. Rev. Entomol.* 53, 361–385.
- Jervis, M.A., Ferns, P.N., Heimpel, G.E., 2003. Body size and the timing of egg production in parasitoid wasps: a comparative analysis. *Funct. Ecol.* 17, 375–383.
- Jervis, M.A., Kidd, N.A.C., Fitton, M.G., Huddleston, T., Dawah, H.A., 1993. Flower visiting by hymenopteran parasitoids. *J. Nat. Hist.* 27, 67–105.
- Kaersgaard, C.W., Holmstrup, M., Malte, H., Bayley, M., 2004. The importance of cuticular permeability, osmolyte production and body size for the desiccation resistance of nine species of Collembola. *J. Insect Physiol.* 50, 5–15.
- Kucera, M., Malmgren, B.A., 1998. Logratio transformation of compositional data—a resolution of the constant sum constraint. *Mar. Micropaleontol.* 34, 117–120.
- Kumarasinghe, N.C., Edirisinghe, J.P., 1987. Oothecal parasites of *Periplaneta americana*: parasitization and development in relation to host age. *Insect Sci. Appl.* 8, 225–228.
- Kumarasinghe, N.C., Edirisinghe, J.P., 1991. A preliminary survey of domiciliary cockroaches and their oothecal parasites in Sri Lanka. *Vidyodaya J. Sci.* 3, 35–43.
- Lee, C.Y., 1997. Medical importance of domiciliary cockroaches. *Sing. Microbiol.* 11, 14–17.
- Lee, C.Y., 2007. Perspective in urban insect pest management in Malaysia. *Vector Control Research Unit, Universiti Sains Malaysia, Penang, Malaysia*.
- Lee, J.C., Heimpel, G.E., 2008. Floral resources impact longevity and oviposition rate of a parasitoid in the field. *J. Anim. Ecol.* 77, 565–572.
- Lei, G., Hanski, I., 1998. Spatial dynamics of two competing specialist parasitoids in a host metapopulation. *J. Anim. Ecol.* 67, 422–433.
- Martel, V., Boivin, G., 2004. Premating dispersion in the egg parasitoid *Trichogramma* (Hymenoptera: Trichogrammatidae). *Environ. Entomol.* 33, 855–859.
- Mazer, C.L., Appel, A.G., 2001. Water loss and desiccation tolerances of longwing butterflies (Lepidoptera: Nymphalidae). *Environ. Entomol.* 30, 631–636.
- McFarland, C.D., Hoy, M.A., 2001. Survival of *Diaphorina citri* (Homoptera: Psyllidae), and its two parasitoids, *Tamarixia radiata* (Hymenoptera: Eulophidae) and *Diaphorocyrtus aligarhensis* (Hymenoptera: Encyrtidae), under different humidities and temperature regimes. *Fla. Entomol.* 84, 227–233.
- Meeh, K., 1879. Oberflächenmessungen des menschlichen Körpers. *Z. Biol.* 15, 425–458.
- Mills, N., 2006. Interspecific competition among natural enemies and single versus multiple introduction in biological control. In: Brodeur, J., Boivin, G. (Eds.), *Trophic and Guild Interactions in Biological Control*. Springer, Dordrecht, The Netherlands, pp. 191–220.
- Minot, M.C., Leonard, D.E., 1976. Effect of temperature, humidity, light, and gravity on the parasitoid *Brachymeria intermedia*. *Environ. Entomol.* 5, 427–430.
- Narasimham, A.U., 1984. Comparative studies on *Tetrastichus hagenowii* (Ratzeburg) and *T. asthenogmus* (Waterston), two primary parasites of cockroach oothecae, and on their hyperparasite *Tetrastichus* sp. (*T. miser* (Nees) group) (Hymenoptera: Eulophidae). *Bull. Entomol. Res.* 74, 175–189.
- Narasimham, A.U., Sankaran, T., 1979. Domiciliary cockroaches and their oothecal parasites in India. *Entomographa* 24, 273–279.
- Packard, G.C., Boardman, T.J., 1999. The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? *Comp. Biochem. Physiol. A* 122, 37–44.
- Pawlowsky-Glahn, V., Egozcue, J.J., 2006. Compositional data analysis and their analysis: an introduction. In: Buccianti, A., Mateu-Figueras, G., Pawlowsky, V. (Eds.), *Compositional Data Analysis in the Geosciences: From Theory to Practice*. Special Publications 264, Geological Society, London, UK, pp. 1–10.
- Pawson, B.M., Gold, R.E., 1993. Evaluating different release strategies for the control of American cockroaches using the egg parasitoid, *Aprostocetus hagenowii*. In: Widley, K.B., Robinson, W.H. (Eds.), *Proceedings of the First International Conference on Insect Pests in the Urban Environment*. BPPC Wheatons, Exeter, UK, pp. 407–413.
- Piper, G.L., Frankie, G.W., Loehr, J., 1978. Incidence of cockroach egg parasites in urban environments in Texas and Louisiana. *Environ. Entomol.* 7, 289–293.
- Reierson, A.A., Rust, M.K., Paine, E., 2005. Control of American cockroaches (Diptera: Blattellidae) in sewer systems. In: Lee, C.Y., Robinson, W.H., (Eds.), *Proceedings of the Fifth International Conference on Urban Pests*. Perniagaan Ph'ng, Malaysia, pp. 141–148.
- Rivero, A., Casas, J., 1999. Incorporating physiology into parasitoid behavioral ecology: the allocation of nutritional resources. *Res. Popul. Ecol.* 41, 39–45.
- Rivero, A., West, S.A., 2002. The physiological costs of being small in a parasitic wasp. *Evol. Ecol. Res.* 4, 407–420.
- Roth, L.M., Willis, E.R., 1954. The biology of the cockroach egg parasite, *Tetrastichus hagenowii* (Hymenoptera: Eulophidae). *Trans. Am. Entomol. Soc.* 80, 53–72.
- Roth, L.M., Willis, E.R., 1960. The biotic associations of cockroaches. *Smithsonian Misc. Coll.* 141, 1–470.
- Rusch, A., Suchail, S., Valantin-Morison, M., Sarthou, J.P., Roger-Estrade, J., 2013. Nutritional state of the pollen beetle parasitoid *Tersilochus heterocerus* foraging in the field. *Biol. Control* 58, 17–26.
- Schilman, P.B., Lighton, J.R.B., Holway, D.A., 2007. Water balance in the Argentine ant (*Linepithema humile*) compared with five common native ant species from southern California. *Physiol. Entomol.* 32, 1–7.
- Sorribas, J., Rodriguez, R., Garcia-Mari, F., 2010. Parasitoid competitive displacement and coexistence in citrus agroecosystems: linking species distribution with climate. *Ecol. Appl.* 20, 1101–1113.
- Steidle, J.L.M., Reinhard, J., 2003. Low humidity as a cue for habitat preference in the parasitoid *Lariophagus distinguendus*. *BioControl* 48, 169–175.
- Strand, M.R., Casas, J., 2008. Parasitoid and host nutritional physiology in behavioral ecology. In: Wajnberg, E., Bernstein, C., van Alphen, J.J.M. (Eds.), *Behavioural*

- Ecology of Parasitoids: From Theoretical Approaches to Field Applications. Blackwell Publishing, Oxford, UK, pp. 113–128.
- Suiter, D.R., Patterson, R.S., Koehler, P.G., 1998. Seasonal incidence and biological control potential of *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) in treehole microhabitats. *Environ. Entomol.* 27, 434–442.
- Tee, H.S., Lee, C.Y., 2013. Feasibility of cold-stored *Periplaneta americana* (Dictyoptera: Blattellidae) oothecae for rearing the oothecal parasitoids *Aprostocetus hagenowii* and *Evania appendigaster* (Hymenoptera: Eulophidae; Evaniidae): effect of ootheca age and storage duration. *Biol. Control* 67, 530–538.
- Tee, H.S., Saad, A.R., Lee, C.Y., 2011a. Population ecology and movement of the American cockroach (Dictyoptera: Blattellidae) in sewers. *J. Med. Entomol.* 48, 797–805.
- Tee, H.S., Saad, A.R., Lee, C.Y., 2011b. Evaluation of *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) for the control of American cockroaches (Dictyoptera: Blattellidae) in sewers and crevices around buildings. *J. Econ. Entomol.* 104, 2031–2038.
- Thoms, E.M., Robinson, W.H., 1987. Distribution and movement of the oriental cockroach (Orthoptera: Blattellidae) around apartment buildings. *Environ. Entomol.* 16, 731–737.
- Tylianakis, J.M., Didham, R.K., Wratten, S.D., 2004. Improved fitness of aphid parasitoids receiving resource subsidies. *Ecology* 65, 658–666.
- van Dijken, M.J., van Alphen, J.J.M., 1998. The ecological significance of differences in host detection behaviour in coexisting parasitoid species. *Ecol. Entomol.* 23, 265–270.
- Visser, B., Ellers, J., 2008. Lack of lipogenesis in parasitoids: a review of physiological mechanisms and evolutionary implications. *J. Insect Physiol.* 54, 1315–1322.
- Visser, B., Le Lann, C., den Blanken, F.J., Harvey, J.A., van Alphen, J.J.M., Ellers, J., 2010. Loss of lipid synthesis as an evolutionary consequence of a parasitic lifestyle. *Proc. Natl. Acad. Sci. U.S.A.* 107, 8677–8682.
- Walters, A.C., Mackay, D.A., 2003. An experimental study of the relative humidity preference and survival of the Argentine ant, *Linepithema humile* (Hymenoptera, Formicidae): comparisons with a native *Iridomyrmex* species in south Australia. *Insect. Soc.* 50, 355–360.
- Weseloh, R.M., 1979. Comparative behavioral responses of three *Brachymeria* species and other gypsy moth parasitoids to humidity and temperature. *Environ. Entomol.* 8, 671–675.
- Willmer, P.G., 1986. Foraging patterns and water balance: problems of optimization for a xerophilic bee, *Chalicodoma sicula*. *J. Anim. Ecol.* 55, 941–962.
- Winston, P.W., Bates, D.H., 1960. Saturated solutions for the control of humidity in biological research. *Ecology* 41, 232–237.
- Yeh, C.C., Mu, C.C., 1994. Observation of the life history of *Evania appendigaster* (L.) (Hymenoptera: Evaniidae) in the laboratory. *Chin. J. Entomol.* 14, 369–378.
- Yoder, J.A., Hoy, M.A., 1998. Differences in water relations among the citrus leafminer and two populations of its parasitoid inhabiting the same apparent microhabitat. *Entomol. Exp. Appl.* 89, 169–173.
- Zhang, L.X., Wu, Z.Q., Fan, J.S., Wang, K.Q., 2010. Reproductive characteristic of female *Tetrastichus hagenowii* (Ratzeburg) (Hymenoptera: Eulophidae). *Acta Entomol. Sin.* 53, 76–81.