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Egg maturation, oosorption, and wing wear in *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae), an egg parasitoid of the glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae)

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ABSTRACT

Egg maturation and oosorption in *Gonatocerus ashmeadi* were investigated in the laboratory and the relationship between hind tibia length (HTL) and <12 h egg load, and wing wear and parasitoid age were determined. *G. ashmeadi* given access to honey-water and hosts, on average, matured 77 eggs in excess of those they were born with. The number of mature eggs in female *G. ashmeadi* provided honey-water with no hosts significantly declined after 163 degree-days eggs, while the number of 'dissolved' eggs (partially disintegrated mature eggs) increased by nine eggs after 163 degree-days. These results are consistent with oosorption. There was a significant positive correlation between HTL and <12 h egg load. The ovigeny index (the number of mature eggs at female emergence divided by potential lifetime fecundity) for *G. ashmeadi* was calculated as 0.22 indicating that this parasitoid is a syn-ovigenic species when studied under laboratory conditions. There was a significant positive correlation between wing wear (measured as the number of broken setae per wing) and parasitoid age in the laboratory. The practical implications of these results for *G. ashmeadi* on the biological control of *Homalodisca vitripennis* are discussed.

1. Introduction

Homalodisca vitripennis (Germar) (Hemiptera: Cicadellidae) (formally *Homalodisca coagulata* (Say) [Takiya et al., 2006]) is an exotic pest in California (USA) after successfully invading in the late 1980's from its native range in the southeastern USA. This insect is an economically significant vector of the xylem-limited bacterium, *Xylella fastidiosa*, which causes disease in a variety of important plants including Pierce's Disease in grapes, almond leaf scorch, alfalfa dwarf, phony peach disease and oleander leaf scorch (Blua et al., 1999; UCOP, 2000; Varela et al., 2001).

Gonatocerus ashmeadi Girault (Hymenoptera: Mymaridae) is a solitary endoparasitoid that parasitizes *H. vitripennis* eggs and has been resident in California since 1978 (Huber, 1988). Genetic studies indicate this parasitoid is native to the southeast USA and probably invaded California with *H. vitripennis* (Vickerman et al., 2004). *G. ashmeadi* is the key natural enemy of *H. vitripennis* egg masses in California providing around 12% and 19% parasitism of spring and summer *H. vitripennis* generations, respectively (Pilkington et al., 2005). Substantial laboratory work has been conducted to parameterize basic aspects of the reproductive biology (Irvin and Hoddle, 2005a,b; Irvin et al., 2006, 2007; Irvin and Hoddle, 2006, 2007), developmental biology (Pilkington and Hoddle, 2006; Chen et al., 2006a), and behavior (Velema et al., 2005; Chen et al., 2006b) of *G. ashmeadi*. Although basic aspects of the biology of *G. ashmeadi* are well understood in the laboratory, it is unknown whether this species is a pro- or syn-ovigenic parasitoid.

Gonatocerus parasitoids are classified as strictly pro-ovigenic (Jervis and Copland, 1996). Chen et al. (2006a) suggested *G. ashmeadi* is pro-ovigenic because female parasitoids showed no pre-mating or pre-ovipositional periods. However, research specifically investigating ovigeny, egg maturation, and oosorption capabilities of *G. ashmeadi* has not been conducted to verify the assumption of pro-ovigeny. Quantification of these basic physiological attributes affecting egg development is needed to better understand ovigeny in *G. ashmeadi* because this basic knowledge could assist in the development of field-based strategies for improving biological control of *H. vitripennis* in California. Therefore, the following research sought to investigate three factors known to affect ovigeny: (1) the relationship between the size of female *G. ashmeadi* and egg load at time of emergence from *H. vitripennis* egg masses; (2) whether oosorption occurs in the absence of hosts, and (3) if female parasitoids are able to develop and mature eggs in excess of those they emerge with. Additionally, the relationship between wing wear and *G. ashmeadi* age was determined in the laboratory. This technique has potential for relatively estimating parasitoid age when

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field captured specimens are examined (Heimpel et al., 1996; Lee et al., 2006). Quantifying these three basic parameters that affect ovigeny in concert with relative age estimates for *G. ashmeadi* will provide fundamental data that could be used to conduct field evaluations to determine life time parasitization rates of *H. vitripennis* eggs by *G. ashmeadi* under prevailing field conditions. Further, better understanding of ovigeny in *G. ashmeadi* could assist in the identification of factors that can be manipulated in the field (e.g., resource provisioning in the context of conservation biological control) to promote the full reproductive potential of this parasitoid which could enhance biological control of *H. vitripennis*.

2. Materials and methods

2.1. Insect colonies and laboratory conditions

Laboratory colonies of *H. vitripennis* and *G. ashmeadi* were maintained at the University of California, at Riverside (UCR). Parasitoid colonies were held at 26 ± 2 °C and 30–40% RH under a L14:10D photoperiod and reared on *H. vitripennis* eggs laid on 'Eureka' lemon leaves, a preferred lemon variety for *H. vitripennis* oviposition and parasitoid foraging (Irvin and Hoddle, 2004; [see Irvin and Hoddle, 2005b for plant maintenance details]). Experiments were conducted in the laboratory at 26 ± 2 °C and 30–40% RH under a L14:10D photoperiod.

2.2. Determining the relationship between hind tibia length and <12 h egg load

Hind tibia length (HTL) as a measure of parasitoid size has been demonstrated to be reliably and positively correlated with parasitoid longevity and fecundity (Jervis et al., 2003). To determine the relationship between HTL and <12 h egg load for *G. ashmeadi, H. vitripennis* and *Homalodisca lacerta* Fowler (a smaller native California congener of *H. vitripennis*) egg masses were collected from citrus and jojoba (*Simmondsia chinensis* [Link] Schneider) at the University of California, Riverside, Agricultural Operations Facility during April–August, 2006, and placed into Petri dishes (9 × 1 cm, Becton Dickinson Labware, Becton Dickinson and Co., Franklin Lakes, NJ) lined with moist filter paper (9 cm Whatman Ltd., International, Maidstone, England). Two mated female *G. ashmeadi* (<48 h old) were placed into each Petri dish for 24 h and allowed to oviposit. The two sharpshooter hosts which have significantly different egg sizes (Al-Wahaibi, 2004) were used to vary the size of emerging *G. ashmeadi* thereby ensuring a range of female sizes to which egg load upon emergence could be correlated. Petri dishes were checked daily for *G. ashmeadi* emergence and recently emerged females (<12 h) were killed by freezing. Females were placed on a microscope slide (25 × 75 × 1 mm, Gold Seal Products, Portsmouth, NH) and the right metathoracic tibia was removed, mounted in Canada balsam, and covered with a glass cover slip (18 × 18 × 2 mm, Fisher Scientific, Pittsburgh, PA). The length of each tibia was measured to within 0.003 mm using an optical scale lens positioned in the eye piece of a compound microscope at 160× magnification. Tibiae were measured from the point of attachment to the femur to the top of the first tarsal segment at the base of the spur. One drop of tap water was applied to the remaining female body, the abdomen was removed using fine forceps, and the ovaries were teased from the abdomen. A glass cover slip was placed over the dissected material and gently pressed to release eggs from the ovaries. The number of mature and immature eggs was counted under a dissecting microscope at 20× and 160× magnification, respectively. Completely mature eggs were distinguished from immature eggs by shape (mature eggs contain a slender tapering pedicel at the anterior end [Sahad, 1982, Fig. 1]) and size (mature eggs: 0.332 ± 0.003 mm; immature: 0.180 ± 0.005 mm; Fig. 1). A total of 376 female *G. ashmeadi* (<12 h old) reared from either *H. vitripennis* or *H. lacerta* eggs were dissected during April 14–August 31, 2006. Egg load data was log-transformed prior to analyses to equalize variances. The relationship between HTL and <12 h egg load was determined for spring (April–June) and summer (July–August) parasitoid generations using linear regression on log-transformed data. The regression equation and means presented here were back-transformed. A two-way ANOVA was performed in SAS (1990) to determine the effect of season, sharpshooter species, and season * species interaction on HTL (data transformed using Box Cox 8 prior to analysis [Cox, 1972]) and egg load data (untransformed data used for analysis).

2.3. Oosorption measurements

Newly emerged mated female *G. ashmeadi* (<12 h old) were held individually, without hosts, in inverted ventilated 130 ml plastic vials (see Irvin and Hoddle, 2005a for details on vial design). A drop of 50% honey-water (Natural uncooked honey, Wild Mountain Brand, Oakland CA) was placed on the lid and renewed daily. Water was provided via a moist cotton ball placed on the ventilated lid of each vial. After 2 h post-emergence (i.e., day 0), 1, 3, 5, 7, 9, 11 and 13 days, between 12 and 20 vial replicates were dismantled and female *G. ashmeadi* were killed and dissected. The right and left forewings were removed (used for Section 2.5 below), the right metathoracic tibia was measured, and the number of mature and immature eggs was counted as previously described. The number of 'dissolved eggs' (mature eggs which had disintegrated; Fig. 1)

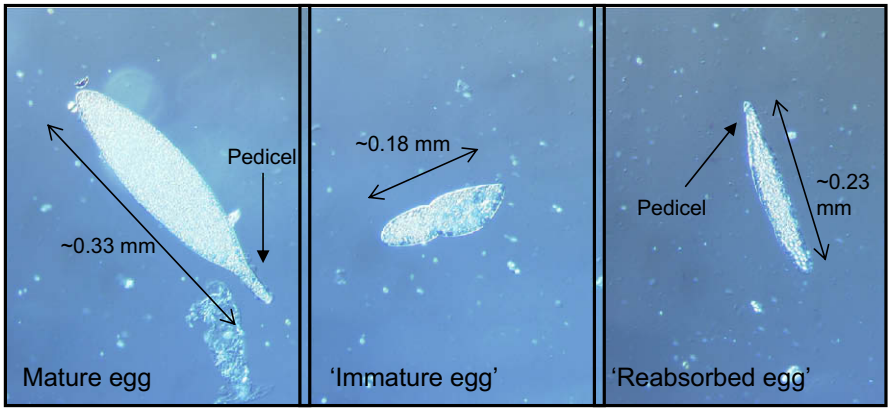

Fig. 1. Mature, non-mature and 'dissolved' eggs dissected from the ovaries of female *Gonatocerus ashmeadi* (all photos at 160× magnification).

was also recorded. Potential fecundity (mature + immature eggs + dissolved eggs) was calculated for each female. HTL was used to predict <12 h egg load of each female by using the linear relationship between HTL and <12 h egg load as described in Section 2.2. Parasitoid age was converted to physiological age using day-degree estimates with the lower temperature threshold for adult development being estimated as 3.41 °C as derived from data reported in Pilkington and Hoddle (2006).

To investigate whether female *G. ashmeadi* continued egg maturation in the absence of hosts, linear regression was used (SAS, 1990) to determine whether the linear relationship between mean potential fecundity and parasitoid age was significantly different from the linear relationship between predicted <12 h egg load and parasitoid age (i.e., comparing potential fecundity to predicted <12 h egg load). To investigate the effect of parasitoid age on different egg stage categories, one-way ANOVA's were conducted on mean number of mature eggs (data were square-root transformed prior to analysis), mean number of immature eggs (square-root transformed), predicted <12 h egg load data (Box Cox 3 transformed) and mean potential fecundity (raw data) in SAS (SAS, 1990). Tukey's Studentized Range test at the 0.05 level of significance was used to separate means. Data for the mean number of dissolved eggs contained a high proportion of zeros for age categories $\leqslant$7 days. Therefore, Poisson regression (Cameron and Trivedi, 1998) was used to determine the effect of age on the number of dissolved eggs. To separate means Tukey's Studentized Range test at the 0.05 level of significance was used to compare data (transformed using Box Cox 0.8) between 9, 11 and 13 days, while for the age categories $\leqslant$7 days, data were converted into binomial data, where "no dissolved eggs = 0" and "some dissolved eggs = 1". Fisher's Exact test (Agresti, 2002) at the 0.05 level of significance was used to separate means for data $\leqslant$7 days. To demonstrate that the number of mature eggs declined significantly later in life and therefore suggesting the possibility of oosorption, linear regression was performed on the number of mature eggs for *G. ashmeadi* aged between 163 and 302 degree-days. Finally, two parabolic models were fitted to the relationship between mature eggs and parasitoid age, and the relationship between immature eggs and parasitoid age. *F*-tests were preformed at the 0.05 significance level to determine goodness of fit of the parabolic models and whether the *x*-square term was significantly different from zero. A Wald test (Fitzmaurice et al., 2004) was used at the 0.05 significance level to investigate the hypothesis that data for mature eggs were the inverse of data collected for immature eggs.

2.4. Egg maturation measurements

Newly emerged mated female *G. ashmeadi* (<12 h old) were held individually in inverted ventilated 130 ml plastic vials and supplied water and a drop of honey-water daily. Approximately 35 *H. vitripennis* eggs <72 h of age laid on 'Eureka' *Citrus limon* leaves were supplied to females in vials by placing leaf stems through holes in the lid of a 130 ml plastic vial filled with deionized water (Irvin and Hoddle, 2005a). The number of host eggs supplied daily was determined from previous fecundity studies for *G. ashmeadi* (Pilkington and Hoddle, 2006; Irvin and Hoddle, 2007). The inverted vial containing female *G. ashmeadi* was attached to the lid of the vial containing host eggs on leaves. *H. vitripennis* egg masses were renewed daily and exposed leaves bearing host egg masses were placed in labeled Petri dishes lined with moist filter paper and left at 26 °C for three weeks for parasitoid emergence. Over this emergence period, leaves sometimes decayed which prevented parasitoid emergence. To prevent data losses from decayed leaves, host eggs were dissected and the numbers of unemerged parasitoids were recorded. Realized fecundity was calculated as the total number of emerged and unemerged parasitoid progeny. After provisionment of host eggs for either 1, 3, 4, 5, 7, 9, 11 or 13 days, between 9 and 20 vial replicates were dismantled for each time interval and female *G. ashmeadi* were killed and dissected. *G. ashmeadi* <2 h of age were dissected to obtain egg load data for day zero of life. The metathoracic tibia was measured and the number of mature and immature eggs was counted as previously described. No dissolved eggs were observed from dissections. Potential fecundity (mature + immature eggs) and predicted <12 h egg load was calculated for each female parasitoid as previously described. Parasitoid age was converted to physiological age using day-degree estimates (see Section 2.3).

One-way ANOVA was used to investigate the effect of parasitoid age on predicted <12 h egg load (raw data), potential fecundity (raw data), realized fecundity (transformed using Box Cox −0.5), the number of mature eggs (raw data) and the number of immature eggs (square-root transformed) in SAS (1990). Tukey's Studentized Range test at the 0.05 level of significance was used to separate means. To determine whether female *G. ashmeadi* continue maturing eggs after emergence in the presence of hosts, paired *t*-tests were used on untransformed data to compare predicted <12 h egg load and potential fecundity at each age category. To determine the number of eggs female *G. ashmeadi* matured in excess of those they emerged with, the difference between predicted <12 h egg load (as predicted by HTL) and potential fecundity was calculated at each parasitoid age and linear regression was used to determine the relationship between parasitoid age and the number of eggs matured after emergence.

The ovigeny index has been defined as the number mature eggs at female emergence divided by potential lifetime fecundity (Jervis et al., 2001). An ovigeny index value for female *G. ashmeadi* was calculated as the mean number of mature eggs on day 0, divided by the average potential fecundity of days 7, 9, 11 and 13 (where potential fecundity had stabilized and there was no significant difference between days).

2.5. Relationship between wing wear and parasitoid age

The right and left forewings were removed from 12 to 20 female *G. ashmeadi* aged 0, 1, 3, 5, 7, 9, 11 and 13 days in the laboratory (see Section 2.3 above). Forewings were mounted in Canada balsam and covered with a glass cover slip. Photographs of each wing were taken using Automontage software at 160× magnification. The number of broken setae around the entire wing margin was counted and the mean total number of setal hairs per female was calculated. Parasitoid age was converted to physiological age using day-degree estimates (Section 2.3). Linear regression was performed in SAS (1990) to determine the relationship between *G. ashmeadi* age and the number of broken setae per female.

3. Results

3.1. Determining the relationship between HTL and <12 h egg load

There was a significant positive correlation between HTL and <12 h egg load for both spring and summer *G. ashmeadi* generations (Fig. 2). The largest *G. ashmeadi*, with HTLs greater than 0.64 mm, had up to 182% more eggs in their oviducts (96 eggs) compared with individuals with HTL of less than 0.42 mm (34 eggs). There was a significant effect of season ($F = 17.50$, df = 1,332, $p < 0.0001$) and host species (large *H. vitripennis* eggs vs. small *H. lacerta* eggs) ($F = 164.62$, df = 1,332, $p < 0.0001$) on the HTL of *G. ashmeadi*, whereas, the interaction between season and host species was not significant ($F = 2.53$, df = 1,332, $p = 0.11$). Similarly, there was a significant effect of season ($F = 6.91$, df = 1,335, $p < 0.01$) and host species ($F = 84.23$, df = 1,335, $p < 0.0001$) on <12 h egg load, and no significant interaction effect ($F = 2.06$, df = 1,335, $p = 0.15$). Female

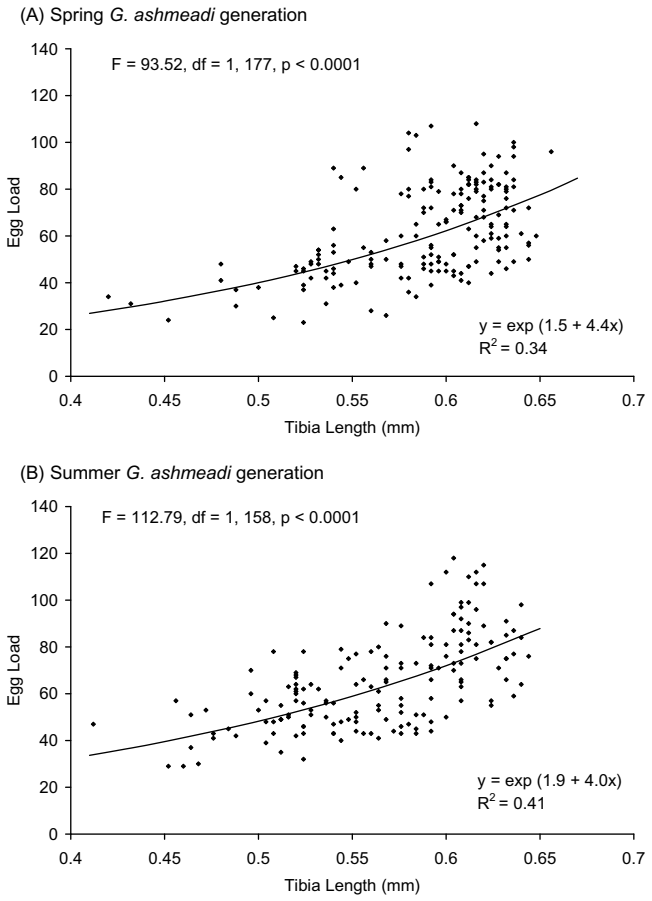

Fig. 2. Relationship between <12 h egg load and female size as measured by hind tibia length for different sized *Gonatocerus ashmeadi* reared from *H. vitripennis* and *H. lacerta* eggs in (A) spring [April 14th–June 30th] and (B) summer [July 1st–August 31st] in 2006.

G. ashmeadi emerging from summer (July 1st–August 31st, 2006) collected *H. vitripennis* egg masses were 0.025 mm smaller, but contained on average 3 eggs more than those emerging from spring (April 14th–June 30th, 2006) egg masses (Table 1). *G. ashmeadi* emerging from *H. vitripennis* eggs were 0.057 mm larger and contained on average 19 eggs more than those females emerging from smaller *H. lacerta* eggs.

3.2. Oosorption measurements

There was no significant effect of age on predicted <12 h egg load ($F = 1.47$, df = 7,113, $p = 0.19$) and mean potential fecundity ($F = 1.48$, df = 7,113, $p = 0.18$) (Fig. 3a). The linear relationship between mean potential fecundity and parasitoid age ($y = 1.0069x + 63.691$; $R^2 = 0.48$) was not significantly ($z = -0.04$, p > 0.05) different from the linear relationship between predicted <12 h egg load and parasitoid age ($y = 0.0763x + 65.703$; $R^2 = 0.01$). Parasitoid age had a significant effect on the number of mature eggs ($F = 25.31$, df = 7,113, $p < 0.0001$), immature eggs ($F = 41.29$, df = 7,113, $p < 0.0001$), and dissolved eggs ($\chi^2 = 306.47$, df = 1, $p < 0.0001$). The number of mature eggs significantly increased by 24 eggs from 0 to 70 degree-days, and decreased by 16 eggs from 209 to 256 degree-days in the absence of hosts (Fig. 3a). This parabolic relationship ($y = 32.3 + 9.4x - 0.7x^2$; goodness of fit test: $F = 69.93$, df = 2,119, $p < 0.0001$; x-squared term significance: $F = 125.70$, df = 1,119, $p < 0.0001$) was not significantly (Wald test: $\chi^2 = 5.99$, df = 2, $p > 0.05$) different from the inverse parabolic relationship between immature egg production and parasitoid age ($y = 30.7 - 7.0x + 0.5x^2$; goodness of fit test: $F = 105.82$, df = 2,119, $p < 0.0001$; x-squared term significance: $F = 147.88$, df = 1,119, $p < 0.0001$) indicating that they were inversely equivalent. Linear regression on the number of mature eggs in female *G. ashmeadi* aged 163–302 degree-days showed that the number of mature eggs significantly declined in female *G. ashmeadi* later in life ($y = -4.0x + 92.4$; $R^2 = 0.36$; $F = 32.41$, df = 1,58; $p < 0.0001$). The number of dissolved eggs counted 0–209 degree-days after *G. ashmeadi* emerged remained statistically equivalent, then increased by nine eggs from 209 to 256 degree-days in the absence of hosts (Fig. 3a).

Table 1
Hind tibia length (HTL) and <12 h egg load of dissected *G. ashmeadi* emerging from *H. vitripennis* and *H. lacerta* egg masses in spring (April 14th–June 30th) and summer (July 1st–August 31st) in 2006 [means ± SEM's; different letters (a,b) indicate significant differences between seasons; different Roman numerals (i,ii) indicate significant differences between sharpshooter species].

	Spring	Summer	*H. vitripennis*	*H. lacerta*
HTL (mm)	0.590 ± 0.003 a	0.565 ± 0.003 b	0.594 ± 0.002 i	0.537 ± 0.003 ii
Egg load	62.19 ± 1.44 a	65.11 ± 1.55 b	68.84 ± 1.25 i	49.84 ± 1.09 ii

3.3. Egg maturation measurements

Parasitoid age had a significant effect on all egg count parameters (Fig 3b; mature eggs: $F = 16.19$, df = 8,108, $p < 0.0001$; immature eggs: $F = 61.53$, df = 8,104, $p < 0.0001$; realized fecundity: $F = 74.52$, df = 8,104, $p < 0.0001$; predicted <24 h fecundity: $F = 6.70$, df = 8,104, $p < 0.0001$; potential fecundity: $F = 23.41$, df = 8,104, $p < 0.0001$). The mean number of mature eggs in female ovaries decreased by 17 eggs from day 0 to 23 degree-days then remained statistically equivalent for the remaining age categories. Similarly, the number of immature eggs decreased by 18 eggs from day 0 to 23 degree-days (Fig. 3b). Realized fecundity increased by 131 eggs from 0 to 302 degree-days. Interestingly, potential fecundity significantly decreased by 11 eggs from 0 to 23 degree-days, and then consistently increased to a maximum of 143 eggs at 256 degree-days (Fig. 3b).

Potential fecundity was not significantly different from predicted <12 h egg load at 0 and 70 degree-days. For 23 degree-days, predicted <12 h egg load was significantly higher (by 26 eggs) than potential fecundity, whereas, for the remaining age categories potential fecundity was statistically higher (up to 77 eggs) than predicted <12 h egg load (Fig. 3b). There was a significant positive correlation between female age and the number of eggs matured by female *G. ashmeadi* after emergence ($y = 0.3x - 9.6$; $R^2 = 0.84$; $F = 36.11$, df = 1,7, p < 0.001). The ovigeny index for laboratory reared *G. ashmeadi* was 0.22, indicating syn-ovigeny under prevailing experimental conditions.

3.4. Relationship between wing wear and parasitoid age

There was a significant positive correlation between parasitoid age and the number of broken setae on the forewings of female *G. ashmeadi* ($F = 39.83$, df = 1,100, $p < 0.0001$; $R^2 = 0.28$) (Fig. 4). The forewings of one day old female *G. ashmeadi* had on average 7.1 ± 1.0 broken setae, whereas, females 13 days of age had 16.8 ± 1.5 broken setae.

4. Discussion

4.1. Relationship between HTL and egg load

HTL accounted for up to 40% of the variance in <12 h egg load indicating that HTL can be used for estimating <12 h egg

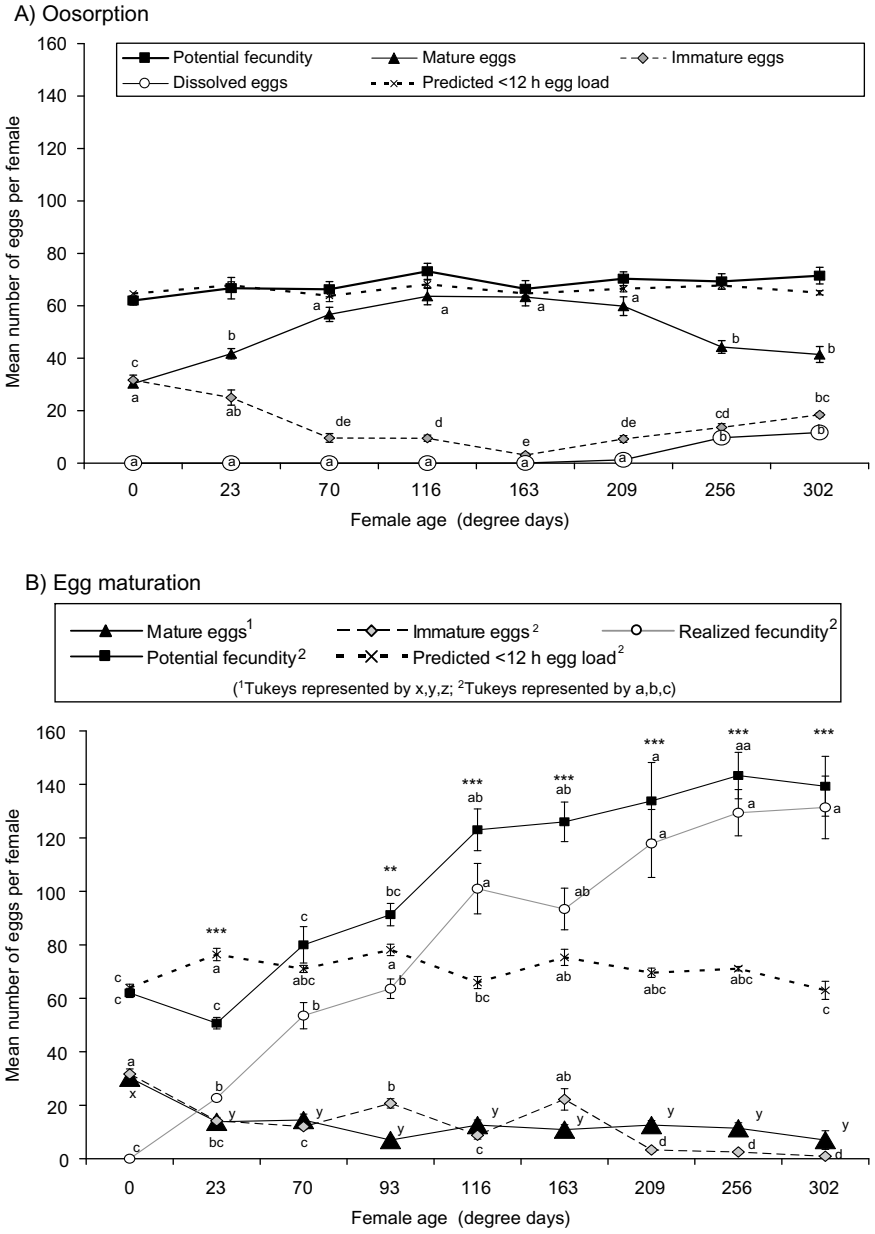

Fig. 3. The effect of age on the mean (±SEM) number of mature eggs, immature eggs, 'dissolved' eggs, potential fecundity (realized fecundity + eggs present in ovaries) and predicted <12 h egg load (estimated from hind tibia length) and realized fecundity of newly emerged female *G. ashmeadi* presented with (A) no hosts and (B) hosts daily for 302 degree-days (13 days at 26 °C) (different letters indicate significant differences between age categories, asterisks in B indicate a significant [$^{**}p < 0.01$, $^{***}p < 0.001$] difference between potential fecundity and predicted <12 h egg load for each age category).

load and reproductive potential of individual *G. ashmeadi.* As with this study, a positive correlation between female size and egg load has been found for other mymarid species (Cronin and Strong, 1996; Jepsen et al., 2007), but this result is not always consistent across mymarid species (Riddick, 2005a). *G. ashmeadi* emerging from *H. vitripennis* egg masses were larger and contained 19 eggs more than those females emerging from smaller *H. lacerta* eggs. Fitness costs associated with utilization of sub-optimal hosts may have important ecological implications for *G. ashmeadi*, by reducing its effectiveness as a biological control agent of *H. vitripennis*. Prior studies have shown that larger female parasitoids are more reproductively successful in the field than smaller females (Kazmer and Luck, 1995; Ellers et al., 1998) and experience reduced risk from environmental challenges (Bartlett, 1962).

4.2. Wing wear

The significant positive correlation between wing wear and parasitoid age indicates that wing wear can be used to estimate the age of female *G. ashmeadi* in the laboratory. However, it is unknown exactly how wing wear estimates for laboratory reared *G. ashmeadi* corresponds to wing wear exhibited by parasitoids living under field conditions. Wing damage in the field is not only a factor of longevity, but also temperature dependent flight, abrasion from the habitat, or predator attack (Allsopp, 1985; Wall and Smith, 1997). Lee et al. (2006) found that *Diadegma insulare* (Cresson) (Ichneumonidae) enclosed in Petri dishes broke wing setae at a slower rate, than conspecifics in field cages. However, they concluded that wing damage observed from field caged parasitoids may not represent parasitoids foraging in the field because caged

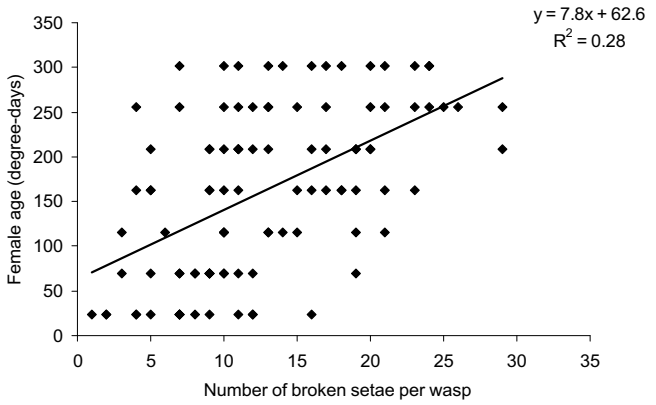

Fig. 4. The relationship between parasitoid age and number of broken setae (hairs) counted on the forewings of female *G. ashmeadi* reared in the laboratory at 26 °C.

parasitoids aggregated in cage corners causing higher levels of wing damage than observed under natural field conditions. Additionally, mark and recapture techniques used for field aging studies (Hagler and Jackson, 2001) may also increase the breakage rate of wing setae (Lee et al., 2006). Consequently, laboratory, field cage, and field methods for estimating parasitoid age have identifiable shortcomings, and it may therefore be difficult to determine in absolute terms how wing wear of laboratory aged *G. ashmeadi* relates to observed wing damage in field populations of *G. ashmeadi*.

However, wing wear indices may be useful in estimating and comparing relative ages between groups of insects within the same environment (Lee and Heimpel, 2008) because high levels of variation in the relationship between parasitoid age and wing wear estimates make it difficult to estimate the absolute age of parasitoids in the field (Heimpel et al., 1996). Alternatively, non-intrusive methods of age grading *G. ashmeadi* such as near infra-red spectroscopy may effectively address questions pertaining to parasitoid age estimation under field conditions (Perez-Mendoza et al., 2004).

4.3. Oosorption, egg maturation and ovigeny

Roberts and Schmidt (2004) reported that mature egg depletion observed in *Venturia canescens* Grav. (Ichneumonidae) in the absence of hosts was a result of females depositing eggs onto the sides of experimental containers. This phenomenon is unlikely in *G. ashmeadi* since the current study detected the presence of 'dissolved eggs' (mature eggs in varying degrees of degradation) in older parasitoids, indicating that expired eggs were being broken down internally and not being discarded externally. The number of dissolved eggs counted in ovaries of female *G. ashmeadi* in the absence of hosts increased by 15 eggs after 163 degree-days suggesting that mature eggs degraded once egg quality declined or a maximum storage capacity was reached.

Some parasitoid species may break down and reabsorb mature eggs enabling them to redirect energy into host seeking, survival, and development of newly matured eggs (Collier, 1995; Rivero-Lynch and Godfray, 1997). Resorption may also serve to maintain a supply of freshly mature eggs (Rivero-Lynch and Godfray, 1997). It is unknown whether *G. ashmeadi* resorbed mature eggs, or if mature eggs merely expired and degraded. It is likely oosorption occurs in *G. ashmeadi*. Firstly, the number of mature eggs in female *G. ashmeadi* significantly declined in the absence of hosts after 163 degree-days. This pattern of mature egg depletion has been reported in a number of studies and is consistent with oosorption (van Lenteren et al., 1987; Collier, 1995; Tran and Takasu, 2000; Asplen and Byrne, 2006). Secondly, Irvin et al. (2007) showed that female *G. ashmeadi* given access to honey-water and no hosts survived 269% longer than those provided with honey-water and hosts for oviposition suggesting that oosorption may have occurred when no hosts were present, thereby allowing additional energy supplies that strongly promoted increased longevity.

Thirdly, females held without hosts showed no significant change in total egg load (mature + immature eggs + partially dissolved eggs) for 302 degree-days. Rosenheim et al. (2000) reported a laboratory study conducted by Sanchez (1994) where a similar result for *Aphelinus asychis* Walker (Aphelinidae) was demonstrated and suggested that egg resorption may occur at the same rate as egg maturation. In the current study, the relationship between mature egg production and parasitoid age was the inverse equivalent to the relationship between immature egg production and parasitoid age. This significant relationship demonstrates that in the absence of hosts, female *G. ashmeadi* continued maturing a proportion of immature eggs until 163 degree-days, after which mature eggs were broken down at a rate equal to the number of immature eggs that were being produced by *G. ashmeadi*. Furthermore, when parasitoids were aged between 163 and 302 degree-days, the number of immature eggs significantly increased by 15 eggs, while the number of dissolved eggs increased by 11 eggs. This result suggests that resources attained from degrading expired mature eggs may have been redirected into producing immature eggs.

If oosorption indeed exists in *G. ashmeadi*, it is obligatory because egg maturation continued in the absence of hosts (Quicke, 1997). In the current study, honey-water was provided as a food source to female *G. ashmeadi* during oosorption experiments. In the total absence of food *G. ashmeadi* survives for just 2.5 days (58 degree-days) (Irvin et al., 2007). Therefore, in the current study it is conceivable that oosorption may have occurred earlier than 116 degree-days in the absence of food. Heimpel et al. (1997) showed that over 36 h, oosorption in *Aphytis melinus* DeBach (Aphelinidae) was absent when females were provided honey, but occurred more rapidly in the absence of food. Further research, such as investigating the ultrastructural changes of eggs over time in the absence of hosts (Asplen and Byrne, 2006), is required to determine whether *G. ashmeadi* can oosorp eggs.

Gonatocerus spp. and other mymarids are reported as being strictly pro-ovigenic (Jervis and Copland, 1996; Cronin and Strong, 1993; Jervis et al., 2001, 2003; Riddick, 2005a) and females emerge from hosts with a full load of mature eggs and do not subsequently mature more eggs as they age (Quicke, 1997). Chen et al. (2006a) suggested *G. ashmeadi* is pro-ovigenic since they found that females have no pre-mating or pre-ovipositional period, two characteristics typical of pro-ovigenic species. However, results from the egg maturation study showed that at 302 degree-days, potential fecundity for *G. ashmeadi* was 77 eggs higher than the predicted <12 h egg load. These results suggest that female *G. ashmeadi* matured more eggs as they parasitized hosts during their lifetime and indicates that this species is not strictly pro-ovigenic as assumed.

It is possible that potential fecundity was underestimated in the egg maturation study because some eggs oviposited by females may be unaccounted for due to superparasitism or early larval death that was not detected by host egg rearing or dissections. The potential fecundity of females given hosts 23 degree-days after emergence was 26 eggs lower than the predicted <12 h egg load suggesting that 26 eggs were unaccounted for possibly due to superparasitism or undetected mortality of parasitoid larvae. Chen et al. (2006b) investigated superparasitism by *G. ashmeadi* at six different host densities and demonstrated that females oviposit on average between 1.2 and 10.4 eggs per *H. vitripennis* egg. In Chen et al. (2006b), superparasitism occurred at low parasitoid:host ratios (1 female *G. ashmeadi*: 25 host eggs), which is similar that used in this study (1:30).

In the absence of hosts, female *G. ashmeadi* did not mature more eggs than what they were predicted to emerge with, whereas, in

the presence of hosts, female *G. ashmeadi* produce 121% more eggs than predicted by <12 h egg load. These results suggest that female *G. ashmeadi* may need to oviposit a proportion of their initial egg load to allow sufficient space in their ovaries for development and storage of new eggs. Results from the oosorption study suggest that, like many other parasitoids (Roberts and Schmidt, 2004), *G. ashmeadi* is unable to completely inhibit oogenesis in the absence of hosts since the number of mature eggs continued to increase up to 116 degree-days in the absence of hosts.

Jervis et al. (2001) calculated ovigeny indices for 638 species of parasitoids and concluded that ovigeny exists on a continuum from strictly pro-ovigenic species to extremely syn-ovigenic species. Jervis et al. (2001) defined strictly pro-ovigenic species as having an ovigeny index of 1, whereas, the index is less than 1 for syn-ovigenic species. The ovigeny index for female *G. ashmeadi* calculated from the egg maturation study was 0.22, indicating that *G. ashmeadi* females emerged with only 22% of their potential egg load available for oviposition into hosts. The overall mean index for parasitic syn-ovigenic species investigated by Jervis et al. (2001) was also 0.22, indicating that *G. ashmeadi* is strongly syn-ovigenic.

Female *G. ashmeadi* do not host feed and exhibit no measurable pre-mating or preoviposition periods (Chen et al., 2006a). Although *G. ashmeadi* shares these two traits of pro-ovigenic species (Jervis et al., 2001), the egg maturation and oosorption evidence, and ovigeny index presented here strongly suggest that *G. ashmeadi* is a syn-ovigenic species when provided access to honey-water and hosts in the laboratory. Further, *G. ashmeadi* has other characteristics reported of syn-ovigenic species such as a relatively long lifespan (Irvin and Hoddle, 2007; Irvin et al., 2007) and an idiobiosic life history strategy (i.e., parasitized hosts do not continue to develop) (Quicke, 1997; Jervis et al., 2001).

This work has demonstrated that *G. ashmeadi* is a syn-ovigenic species, a finding that contradicts current reproductive classification of *Gonatocerus* parasitoids (Jervis and Copland, 1996). Other workers have demonstrated that supposedly pro-ovigenic mymarid species can mature additional eggs after emergence from hosts, resulting in conclusions that these species exhibit synovigeny (Carbone and Rivera, 2003; Riddick, 2005b). It appears that the classification of mymarid and *Gonatocerus* parasitoids in particular, as being strictly pro-ovigenic requires reevaluation.

4.4. Application of work

Elucidation of syn-ovigeny in *G. ashmeadi*, the key natural enemy of *H. vitripennis* in California, has provided fundamental information into an underlying mechanism that affects egg limitation in this parasitoid. These data are critical for designing experiments to estimate mortality rates inflicted by individual female parasitoids in the field, and for providing insight as to how to develop conservation biological control programs in highly managed agro-ecosystems to reduce the constraints of egg limitation in a syn-ovigenic species.

Quantification of natural enemy impacts in the field is extremely important for understanding the magnitude to which pest populations are regulated by biological control agents. Even greater insight can be achieved if the life time contributions of individual natural enemies, such as female parasitoids, to pest suppression can be determined in the field. This fine level of resolution is needed for *G. ashmeadi* to better understand the contributions this parasitoid is making to the long-term management of *H. vitripennis* in California. Data collected for *G. ashmeadi* in this study may be able to provide a starting point from which experiments can be designed to estimate the realized life time fecundity of individual *G. ashmeadi* females attacking *H. vitripennis* under field conditions. Estimates of realized life time fecundity for syn-ovigenic parasitoids in the field have been determined for species other than *G. ashmeadi*, (Casas etal., 2000; Jepsen etal., 2007; Lee and Heimpel, 2008).

To more effectively manage *H. vitripennis* with *G. ashmeadi* in California is going to require an increased reliance on conservation efforts, rather than ongoing importations of additional exotic parasitoid species within the framework of a classical biological control program. In some countries (e.g., Tahiti [Grandgirard et al., 2008]) *G. ashmeadi* has provided spectacular control of *H. vitripennis* with egg parasitism rates exceeding 95% year round. In direct contrast, biological control of *H. vitripennis* by *G. ashmeadi* in California has not been as strong and annual egg parasitism rates average around 20% (Pilkington et al., 2005).

A major reason for this discrepancy was the availability of *H. vitripennis* egg masses which were extremely abundant and present year round in Tahiti because of a mild tropical climate, but are rarer in California and even absent over winter because low temperatures inhibit egg laying by overwintering adult *H. vitripennis*. This host free period in California is responsible for a pronounced decline in parasitoid abundance, and is a major constraint on the ability of populations of *G. ashmeadi* to increase quickly in spring to suppress resurgent pest populations resulting from oviposition by adult *H. vitripennis* that successfully overwintered and commenced reproduction in natural enemy free space.

For syn-ovigenic parasitoids, egg maturation continues throughout adult life (as shown here for *G. ashmeadi*) and an adequate food supply is necessary for optimal egg maturation and longevity (Heimpel et al., 1997) which increases fitness by reducing the constraints of egg limitation and maximizing time available for host searching (Heimpel and Rosenheim, 1998). Previous studies by Irvin and Hoddle (2007) showed that female *G. ashmeadi* survived up to 405% longer and produced up to 378% more progeny when fed suitable food sources in the laboratory, and the results of this study indicate that the reason for positive outcomes from resource provisionment is the syn-ovigenic nature of *G. ashmeadi*. Consequently, biological control of *H. vitripennis* by *G. ashmeadi* could be improved by exploiting its syn-ovigenic qualities to increase longevity and fecundity. Maximizing the fitness of *G. ashmeadi*, especially during periods of host scarcity, could be achieved by providing flowering cover crops to provide food and shelter, a strategy that has been demonstrated to improve pest control (Nicholls et al., 2000; English-Loeb et al., 2003; Gurr et al., 2004).

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