

The effect of intraspecific competition on progeny sex ratio in *Gonatocerus* spp. for *Homalodisca coagulata* egg masses: Economic implications for mass rearing and biological control

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Abstract

The effect of the number of simultaneously ovipositing females on progeny sex ratio of three *Homalodisca coagulata* egg parasitoids, *Gonatocerus ashmeadi*, *G. triguttatus*, and *G. fasciatus* was investigated in the laboratory. When one female *Gonatocerus* parasitoid was present, progeny production was strongly female biased producing ~1 male:8 females, 1:14 and 1:9 for *G. ashmeadi*, *G. triguttatus* and *G. fasciatus*, respectively. Increasing the number of simultaneous ovipositing females from 1 to 3, significantly increased percentage of male offspring by up to 61% for all three *Gonatocerus* species. Overall percentage male offspring ranged from 31–34%, and did not significantly differ among species. Overall mean percentage parasitism by *G. ashmeadi* was up to 16% higher compared with *G. triguttatus* and *G. fasciatus*, whereas, average clutch size and mean progeny production was more than twofold higher for *G. fasciatus* when compared with *G. ashmeadi* and *G. triguttatus*. Additional laboratory trials with only *G. ashmeadi* demonstrated that when *H. coagulata* egg masses consisted of 2–13 eggs, 67–100% of females allocated one male offspring per egg mass. When one egg mass was presented to five female *G. ashmeadi* searching concurrently, male offspring was up to 22% higher compared with male offspring from host eggs exposed to one female *G. ashmeadi*. Experiments indicated that allocation of male eggs was not influenced by constant female-to-female contact for 19 h prior to host exposure, and female *G. ashmeadi* did not alter allocation of male eggs when sibling and non-sibling conspecifics were contesting host egg masses. The economic significance for mass rearing is substantial. For *G. ashmeadi* at 2003 production costs and under optimal conditions, estimated cost per female was \$1.42, under suboptimal conditions, cost per female increased to \$4.51; a 218% increase in production cost per female parasitoid.

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Keywords: Biological control; *Gonatocerus ashmeadi*; *Gonatocerus fasciatus*; *Gonatocerus triguttatus*; *Homalodisca coagulata*; Intraspecific competition; Local mate competition; Mass rearing; Sex ratio

1. Introduction

The glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), established in California, USA in the late 1980s, and is creating serious new economic problems in both agricultural and urban landscapes. As this insect continues to spread throughout California, feeding from xylem tissue of over 100 known host plants (Hoddle

et al., 2003), it readily vectors a xylem-dwelling bacterium, *Xylella fastidiosa* Wells et al. (Adlerz, 1980). Different strains of this bacterium induce scorch-like disease in over 100 host plants including grapes, almonds, plums, peaches, alfalfa, oleander and liquidambar (Purcell and Saunders, 1999; Costa et al., 2000; Hopkins and Purcell, 2002). In 2001, the California Department for Food and Agriculture (CDFA) initiated a classical biological control program for *H. coagulata* to reduce densities of this pest and the associated spread of *Xylella*-related diseases. Mymarid egg parasitoids, *Gonatocerus ashmeadi* Girault, *G. triguttatus* Girault, and *G. fasciatus* Girault have been imported,

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mass-reared and released in California as part of this biological control program. *G. ashmeadi* has been resident in California since 1978 (Huber, 1988), while *G. triguttatus* was introduced from Texas in 2001. In 2002, *G. fasciatus* from Louisiana was moved through quarantine and cleared for release in California (CDFA, 2003). *G. ashmeadi* and *G. triguttatus* are solitary endoparasites while *G. fasciatus* is a gregarious endoparasitoid (Triapitsyn et al., 2003).

Gonatocerus ashmeadi, *G. triguttatus* and *G. fasciatus* produce female biased sex ratios of up to 79, 77 and 96s, respectively (Triapitsyn et al., 2003; Irvin and Hoddle, 2005a; Irvin and Hoddle, 2005b). All three parasitoid species are haplodiploid and can manipulate offspring sex ratio by storing sperm from a mating and controlling the access of sperm to eggs at the time of oviposition (Flanders, 1965; Green et al., 1982). Local mate competition (LMC) (Hamilton, 1967) proposes that female parasitoids should always attempt to maximize their reproductive fitness so when a single female visits a patch of hosts, her fitness is proportional to the number of fertilized daughters emigrating from the patch (Green et al., 1982; Hardy, 1992; Hardy et al., 1998). Consequently, the foundress should oviposit just enough haploid sons in each patch to insure her diploid daughters are inseminated. This maximizes female fitness, ensures sufficient males are present to mate with females, and reduces competition between brothers for sib-mating with sisters (Luck et al., 2001).

When the number of ovipositing females accessing a host patch increases, LMC predicts that an increasing investment in sons versus daughters will be advantageous because sons will distribute a greater number of genes to females from unrelated lineages thereby increasing the fitness of their own lineage (Grafen, 1984; Luck et al., 1999; Luck et al., 2001). Consequently, this phenomenon of increasing male production with increasing densities of ovipositing females can have detrimental consequences for mass rearing programs which aim to maximize the production of females for colony maintenance and release as biological control agents (Waage, 1982; Waage et al., 1984; Hall, 1993). This is particularly relevant for *H. coagulata* which is extremely difficult to economically mass rear, and almost impossible to maintain in colony year round. Together, these two factors make egg masses from this pest extremely valuable for parasitoid rearing. Therefore, identifying and understanding factors influencing sex ratio of *Gonatocerus* spp. is imperative for maintaining successful and efficient mass rearing programs and for interpreting parasitoid offspring production from host patches under field conditions (Waage et al., 1984; Waage, 1986). Here, we investigate the effect of female density on progeny sex ratio of three *H. coagulata* egg parasitoids, *G. ashmeadi*, *G. triguttatus* and *G. fasciatus*. The results of these experiments are explored further in two experiments using *G. ashmeadi*, the dominant parasitoid of *H. coagulata* in California (Pilkington et al., 2005). The first of these examined the effect of female-to-female contact prior to host exposure on sex ratio and investigated whether *G. ashmeadi* females rec-

ognized sibling-conspecifics during oviposition. The second study examined the effect of size of egg masses on progeny sex ratio. It was hypothesized that *Gonatocerus* spp. would increase male progeny production in response to increasing female density, as predicted by LMC. It was also hypothesized according to LMC that when *G. ashmeadi* is presented with one egg mass of varying size, one son would be allocated to a specific number of female offspring and this ratio of male to female offspring would be constant across host egg masses of varying sizes.

2. Materials and methods

2.1. Insect colonies

Laboratory colonies of *H. coagulata*, *G. ashmeadi*, *G. triguttatus* and *G. fasciatus* were maintained at the University of California, at Riverside. Parasitoid colonies were held at $26 \pm 2^\circ\text{C}$ and 30–40% RH under a L14:10D photoperiod and reared on *H. coagulata* eggs laid on 'Eureka' lemon leaves, a preferred lemon variety for *H. coagulata* oviposition and parasitoid foraging (Irvin and Hoddle, 2004; [see Irvin et al., 2006 for plant maintenance details]). All experiments were conducted in the laboratory at $26 \pm 2^\circ\text{C}$ and 30–40% RH under light intensity of 1.2 ± 0.2 log lumens/m² and L14:D10 photoperiod.

2.2. Progeny sex ratio resulting from 30 *H. coagulata* eggs

Lemon leaves containing ~30 *H. coagulata* eggs were placed through holes drilled through the lid of a 130 ml plastic vial (40 dram Plastic Vial, Thornton Plastics, Salt Lake City, UT) filled with deionized water and 3 ml of anti-septic (Listerine Antiseptic Mouthwash, Pfizer Inc., New York, NY) to prevent bacterial rot. Eggs were approximately 24–48 h of age (shown to be successfully utilized by all three *Gonatocerus* spp. [Irvin and Hoddle, 2005a]). A second 130 ml plastic vial with ventilation (three 2 cm holes [one on the bottom, and one on each of two sides] covered with mesh netting [80 μm Jelliff Corporation, Southport, CT]) was inverted and attached to the lid of the vial holding the water and lemon leaves. A drop of honey-water (3:1 Natural uncooked honey, Wild Mountain Brand, Oakland, CA) was placed on the side of the inverted vial that covered the test material and one newly emerged male and female parasitoid (~24 h old) were introduced. This procedure was replicated approximately 15 times for each of five female densities for each parasitoid species. Female densities tested were 1, 2, 3, 4, and 5 *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus*. For each female density tested, one male parasitoid of the same species was simultaneously present in test vials to ensure experimental females were mated. Vials containing varying densities of parasitoids were held for 24 h after which parasitoids were removed and vials containing leaves were placed at $26 \pm 2^\circ\text{C}$ and 30–40% RH under a L14:10D for three weeks to allow insects to emerge. Vials were topped up with deionised water every second day and the

numbers of emerged male and female parasitoids for each density of searching females were recorded. Premature drying of leaves sometimes occurred which occasionally prevented successful insect emergence. Therefore, unemerged eggs were dissected and the numbers of unemerged male and female parasitoids were also recorded. Percentage parasitism (number of *H. coagulata* eggs parasitized/total number of eggs) and offspring sex ratio (see below for calculation) was calculated for each female density and parasitoid species. *G. ashmeadi* and *G. triguttatus* are solitary species producing one offspring per *H. coagulata* egg, whereas, *G. fasciatus* is gregarious, and produces multiple offspring per host egg. Consequently, the average clutch size for *G. fasciatus* was also calculated for each female density tested.

Offspring sex ratio was calculated as the total number of male parasitoids (counts of emerged and unemerged parasitoids were combined) divided by the total number of male and female parasitoids combined, and was represented as percentage male offspring. Percentage parasitism and percentage male offspring data were square root arc sine transformed and compared among female densities within species using analysis of variance (ANOVA) in SAS (1990). Tukey's Studentized Range test at the 0.05 level of significance was used to separate means. Mean numbers of male and female parasitoids were compared among female densities for each species using non-parametric Kruskal–Wallis test at the 0.05 level of significance in SAS (1990). Clutch size was compared among female densities for *G. fasciatus* using ANOVA in SAS (1990).

Additionally, percentage parasitism, percentage male progeny, total male and female offspring, and clutch size data were pooled across female densities and means were compared among species using Kruskal–Wallis tests at the 0.05 level of significance in SAS (1990). Logistic regression analyses were used to determine the relationship between female density and percentage male progeny, and whether female density affected percentage parasitism. For percentage male progeny, regression slopes were tested among species at the 0.05 level of significance (SAS, 1990).

2.3. Investigating mechanisms underlying sex allocation in *G. ashmeadi*

Five treatments were set up consisting of: (1) vials containing one female *G. ashmeadi* held in isolation for ~19 h prior to being offered one *H. coagulata* egg mass; (2) vials containing five female non-sibling *G. ashmeadi* held for ~19 h prior to being simultaneously offered one *H. coagulata* egg mass; (3) vials containing five non-sibling female *G. ashmeadi* held for ~19 h prior to being separated and placed individually in vials containing one *H. coagulata* egg mass per female; (4) vials containing five female sibling *G. ashmeadi* held for ~19 h prior to being simultaneously offered one *H. coagulata* egg mass; and (5) a vial containing five sibling female *G. ashmeadi* held for ~19 h prior to being

separated and placed individually in vials containing one *H. coagulata* egg mass per female.

Treatments were replicated 10–26 times and newly emerged mated naïve female *G. ashmeadi* (~48 h of age at time of exposure to egg masses) were supplied honey-water (see above) while in vials during conditioning prior to experimental use. *H. coagulata* egg masses, laid on 'Eureka' lemon leaves and 1–3 days of age (a preferred age range for *G. ashmeadi* [Irvin and Hoddle, 2005a]), contained 14–16 eggs and were exposed to females in each treatment for 2 h. Parasitoids were then removed and vials containing leaves with egg masses exposed to parasitoids were held for three weeks to allow parasitoids to emerge. Females who were not mated (i.e., produced male only progeny) were excluded from analyses. The percentage of male progeny was calculated for each treatment vial, square root arc sine transformed, and compared between treatments using a one-way ANOVA in SAS (1990). Tukey's Studentized Range test at the 0.05 level of significance was used to separate means.

2.4. *Gonatocerus ashmeadi* progeny sex ratio resulting from one host egg mass

One *H. coagulata* egg mass, laid on 'Eureka' lemon and containing a known number of eggs (1–3 days of age), was placed in a double inverted vial as described above with one newly emerged mated naïve female *G. ashmeadi*. Females (~48 h of age at the time of egg exposure) were isolated individually in vials containing one drop of honey-water for ~19 h prior to experimental use. This procedure was replicated 11–24 times for egg mass size category (2–4, 5–7, 8–10, 11–13, 14–16, 17–19, and 20–24 eggs). One (for egg masses sized 2–13 eggs), two (14–19 eggs) or three hours (20–24 eggs) after exposure, parasitoids were removed and vials containing leaves with egg masses exposed to parasitoids were held for three weeks to allow parasitoids to emerge. Exposure times were selected based on preliminary studies in order to maximize foraging time and prevent possible superparasitism. Mean percentage male progeny was calculated for each size category and the r^2 -value determined for an inverse-exponential relationship between percentage male progeny and number of eggs parasitized by female *G. ashmeadi*. The frequency of male progeny per egg mass was calculated for each egg mass size category.

3. Results

3.1. Progeny sex ratio resulting from 30 host eggs

For all three species, percentage male offspring increased significantly with an increase in female density (Fig. 1). When one and two female *G. ashmeadi* were present per vial, up to 61% less male offspring was produced compared with vials containing female densities 3, 4, and 5. These three *G. ashmeadi* treatments did not differ significantly in percentage male progeny (Fig. 1). Placing 1 female *G. triguttatus* per vial resulted in up to 22% less male offspring

compared with the other four female densities, while percentage male progeny did not significantly vary between female densities 2, 3, 4, and 5 (Fig. 1). Increasing the number of foraging *G. fasciatus* females from 1 to 5 per vial, increased percentage of male offspring by 45% (Fig. 1).

When one female *G. ashmeadi*, *G. triguttatus* or *G. fasciatus* was present, progeny production was strongly female biased producing ~1 male:8 females, ~1:14 or ~1:9, respectively (Fig. 1). For *G. ashmeadi*, the total number of male and female offspring significantly varied among female

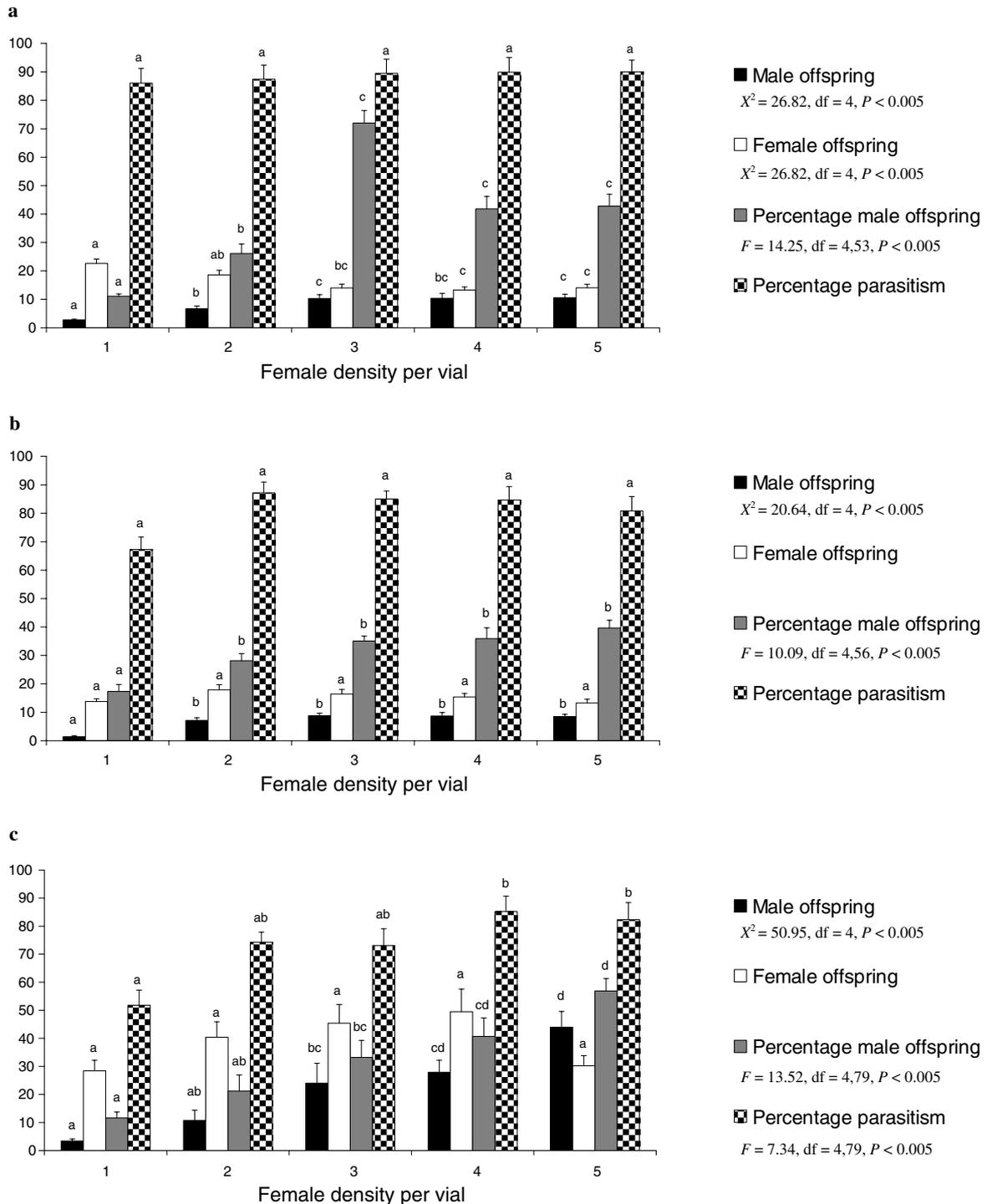


Fig. 1. Percentage parasitism, percentage male offspring and total numbers of emerged and unemerged male and female progeny for (a) *Gonatocerus ashmeadi*, (b) *G. triguttatus*, and (c) *G. fasciatus*, when *Homalodisca coagulata* egg masses were exposed to increasing number of ovipositing females in the laboratory [different letters indicate significant ($P < 0.05$) differences among female densities for each species. Test statistics indicate significant results across female densities for each species; error bars indicate \pm SEM].

densities (Fig. 1). Increasing *G. ashmeadi* female density from 1 to 3 per vial significantly increased male offspring and decreased female offspring by approximately 8 and 9, respectively. For *G. triguttatus*, there was no significant effect of female density on the mean number of female *G. triguttatus* offspring, whereas, the mean number of male progeny was significantly lower when 1 female *G. triguttatus* was present, compared with the other four female densities (Fig. 1). For *G. fasciatus*, male offspring significantly varied among female densities, whereas, there was no significant difference in female *G. fasciatus* offspring among female densities (Fig. 1). Increasing the number of ovipositing *G. fasciatus* from 1 to 5, significantly increased progeny production by 41 male offspring.

There was no significant effect of female density on percentage parasitism by *G. ashmeadi* and *G. triguttatus*. The number of ovipositing females had a significant effect on percentage parasitism by *G. fasciatus*. Increasing female density from 1 to 5 per vial, consistently increased percentage *G. fasciatus* parasitism by 30% (Fig. 1).

Gonatocerus fasciatus average clutch size ranged from 2.1 ± 0.1 to 2.5 ± 0.2 progeny per *H. coagulata* egg and did not statistically differ among female densities.

3.2. Results of pooling data across all female densities for each parasitoid species

Pooling data across all female densities showed that species had a significant effect on numbers of male offspring, numbers of female offspring, percentage parasitism and average clutch size (Fig. 2). In contrast, overall percentage of male offspring for *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* was on average 32% and did not differ significantly across species (Fig. 2). Vials containing *G. fasciatus* resulted in male progeny over twofold higher compared with *G. ashmeadi* and *G. triguttatus*, whereas, production of male

offspring was equivalent between *G. ashmeadi* and *G. triguttatus* (Fig. 2). Mean number of female *G. fasciatus* progeny was twofold higher, compared with *G. ashmeadi* and *G. triguttatus*, while the number of female offspring was equivalent between *G. ashmeadi* and *G. triguttatus* (Fig. 2). Overall mean percentage parasitism by *G. ashmeadi* was significantly higher when compared with *G. triguttatus* and *G. fasciatus*. There was no significant difference in percentage parasitism between *G. triguttatus* and *G. fasciatus* (Fig. 2). Finally, average clutch size (i.e., mean number of offspring produced per *H. coagulata* egg) was over twofold higher for the gregarious *G. fasciatus*, compared with the solitary species *G. ashmeadi* and *G. triguttatus* (Fig. 2).

Logistic regression analyses indicated that percentage male offspring was significantly positively correlated with female density for all three *Gonatocerus* species (*G. ashmeadi*: $\chi^2 = 72.78$, $df = 1$, $P < 0.005$; *G. triguttatus*: $\chi^2 = 22.83$, $df = 1$, $P < 0.005$; and *G. fasciatus*: $\chi^2 = 452.69$, $df = 1$, $P < 0.005$). Percentage male *G. fasciatus* offspring was more positively correlated with female density compared with *G. ashmeadi* ($\chi^2 = 16.12$, $df = 1$, $P < 0.005$) and *G. triguttatus* ($\chi^2 = 41.49$, $df = 1$, $P < 0.005$). The percentage of male *G. triguttatus* offspring was less positively correlated to female density compared with *G. ashmeadi* ($\chi^2 = 5.11$, $df = 1$, $P < 0.05$).

Percentage parasitism by *G. ashmeadi* was not correlated to female density ($\chi^2 = 1.34$, $df = 1$, $P = 0.25$). However, this relationship was positively correlated for *G. triguttatus* ($\chi^2 = 8.66$, $df = 1$, $P < 0.01$) and *G. fasciatus* ($\chi^2 = 180.51$, $df = 1$, $P < 0.005$).

3.3. Investigating mechanisms underlying sex allocation in *G. ashmeadi*

There was no significant difference in percentage male progeny produced between sibling and non-sibling

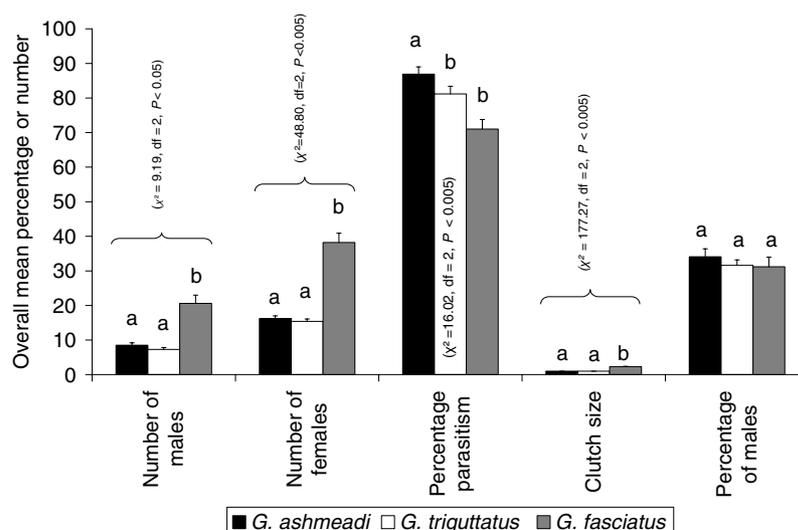


Fig. 2. Mean percentage male offspring, mean numbers of male and female parasitoid offspring, average clutch size and mean percentage parasitism by *Gonatocerus ashmeadi*, *G. triguttatus*, and *G. fasciatus*, pooled across five female densities [different letters indicate significant ($P < 0.05$) differences among species; test statistics for each parameter are indicated; error bars indicate \pm SEM].

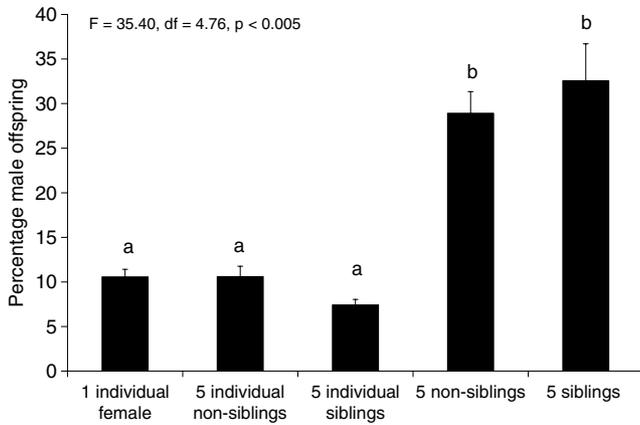


Fig. 3. Mean percentage male progeny produced when one *Homalodisca coagulata* egg mass was exposed to five different treatments containing *Gonatocerus ashmeadi* for 2 h in the laboratory [different letters indicate significant ($P < 0.05$) differences between treatments; test statistic is indicated; error bars indicate \pm SEM].

treatments (Fig. 3). Vials containing one individual female *G. ashmeadi* and vials holding individual females that had been held in groups of five for 19 h prior to host exposure were equivalent. These treatments produced 7–11% male offspring. In contrast, vials containing five female *G. ashmeadi* (siblings and non-siblings) produced up to 22% more male progeny compared to vials holding an individual female *G. ashmeadi* (Fig. 3).

3.4. *Gonatocerus ashmeadi* progeny sex ratio resulting from one egg mass

Mean percentage male progeny was inverse-exponentially correlated with the mean number of eggs parasitized by female *G. ashmeadi* (Fig. 4). Frequency graphs demonstrated that 67–100% of females laid one male offspring per egg mass when *G. ashmeadi* parasitized 2–13 eggs. When egg masses were comprised of 17–19 eggs, 73% of females laid around two male offspring per *H. coagulata* egg mass (Fig. 5).

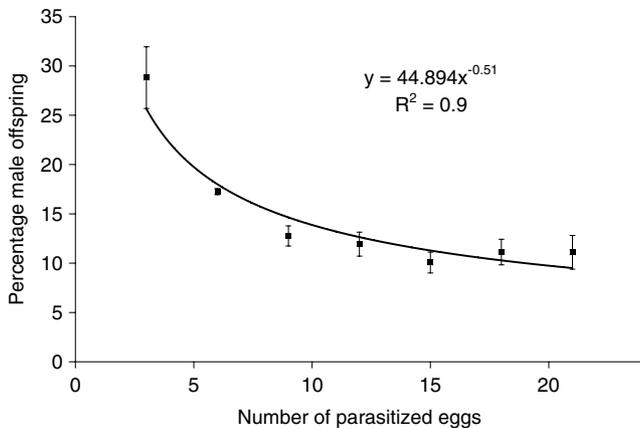


Fig. 4. Correlation between mean percentage male *Gonatocerus ashmeadi* progeny produced and number of *Homalodisca coagulata* eggs parasitized when one female was presented one egg mass of varying size in the laboratory (error bars indicate \pm SEM).

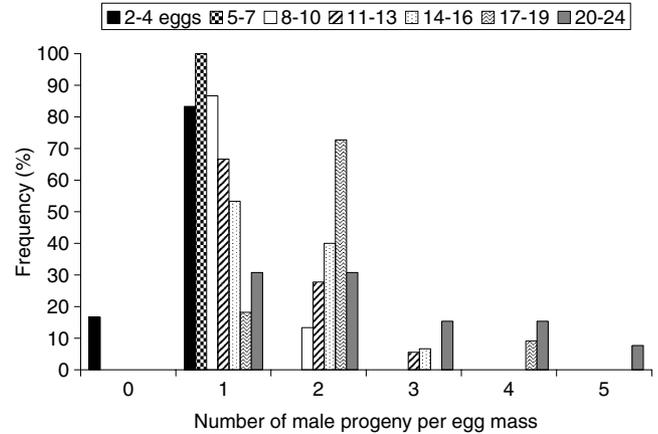


Fig. 5. Frequency of male progeny produced when one female *Gonatocerus ashmeadi* was presented one *Homalodisca coagulata* egg mass of varying size in the laboratory.

4. Discussion

4.1. Sex ratio allocation in *Gonatocerus* spp.

Overcrowding of ovipositing *Gonatocerus* spp. females during mass rearing of parasitoids for biological control releases may adversely affect progeny sex ratio by resulting in more males than necessary to inseminate females and fewer females for colony maintenance and release in the field (Triapitsyn et al., 2003). Therefore, identifying and understanding factors influencing sex ratio of natural enemies is imperative for maintaining successful and economically efficient mass rearing programs (Waage et al., 1984; Waage, 1986). Under optimal conditions, *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* typically produce female biased sex ratios with up to 79, 77, and 96% of progeny being female, respectively (Triapitsyn et al., 2003; Irvin and Hoddle, 2005a,b). However, the results from work presented here indicate that when host density is fixed (i.e., 30 *H. coagulata* eggs per egg mass) and the density of female *Gonatocerus* spp. is increased from 1 to 3 per vial, the percentage of male offspring is significantly increased by up to 61% for all three *Gonatocerus* species.

There are two possible explanations for this observed outcome. First, female *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* may lay fewer eggs when conspecifics are exploiting the same patch. This could occur because in haplodiploid parasitoids male eggs are typically laid first followed by female eggs (Waage, 1982). A reduced number of oviposited eggs due to detection of parasitized hosts (Holmes, 1972; Wylie, 1973; Waage et al., 1984) or interference from ovipositing conspecifics (Jackson, 1966; Wylie, 1971, 1979) could result in the deposition of proportionately more male progeny to female offspring and a more male biased sex ratio results because of this reduced oviposition sequence (Antolin, 1992).

Alternatively, the increase in male progeny in response to increasing ovipositing female density may be attributable to

LMC since this model predicts an increasing investment in sons versus daughters under these conditions (Werren, 1980; Grafen, 1984; Luck et al., 1999, 2001). It is hypothesized under LMC theory that female parasitoids always attempt to maximize their fitness (Hamilton, 1967). Under this assumption, the first female to parasitize a group of hosts would bias her progeny sex ratio towards females. The second female exploiting the same patch would benefit by ovipositing a greater proportion of male offspring to fertilize daughters deposited by the first female thus increasing the fitness of her lineage by reducing the rates at which sib-matings occur and increasing outcrossing (Luck et al., 2001). Additionally, LMC requires sib-mating on or near the host from which they emerge (e.g., Waage and Ng, 1984; Suzuki and Hiehata, 1985; Kazmer, 1992). Male *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* all emerge one day prior to females and remain close to the parasitized egg mass from which they emerged, and they immediately begin mating with newly emerged females (Triapitsyn et al., 2003; Irvin personal observation). This may suggest that these *Gonatocerus* species exhibit LMC, however, additional research into the sequence of sex allocation is required to resolve this issue.

4.2. Sex ratio trends for *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus*

When 3, 4, and 5 female *G. ashmeadi* were present per vial, percentage of male offspring remained statistically equivalent among female densities suggesting that increasing female density beyond five per host patch is unlikely to further increase the proportion of male offspring. The increased number of ovipositing *G. ashmeadi* increased the proportion of male progeny until sex ratio reached an equilibrium of approximately 42%, which is similar to the maximum sex ratio of 50% predicted by the Hamilton's (1967) LMC model. *G. triguttatus* showed similar results since progeny sex ratio stabilized around an equilibrium of 40%, when female density was increased beyond 2 per vial.

Results presented here for *G. fasciatus* were consistent with Triapitsyn et al. (2003) who found that a sex ratio of 1:9 was obtained from original rearing colonies from Louisiana. Increasing female density from 1 to 5 in the current study significantly increased male *G. fasciatus* offspring, while the number of female offspring remained constant. This result may have occurred because gregarious parasitoids often deposit male progeny in already parasitized hosts (Waage, 1986). Additionally, as female density increased, percentage parasitism by *G. fasciatus* also increased which was solely attributable to an increase of 41 male progeny, while female numbers remained statistically invariable. Furthermore, this substantial increase in male offspring may result in intense intraspecific larval competition and reduction in the size of females. Although results showed that female density had no significant effect on *G. fasciatus* clutch size, Triapitsyn et al. (2003) observed that overcrowding in *G. fasciatus* cultures resulted in

smaller and presumably less fit adults emerging from each *H. coagulata* egg.

4.3. Investigating mechanisms underlying sex allocation in *G. ashmeadi*

The present study also sought to investigate the mechanisms behind changes in *G. ashmeadi* sex ratio allocation in response to female density. When vials containing five female *G. ashmeadi* were exposed to one egg mass (~15 eggs), more male offspring were produced compared with those vials holding one individual female *G. ashmeadi*, thereby supporting results obtained from the first experiment (exposure to ~30 host eggs). Results also showed that *G. ashmeadi* male progeny production was comparable between vials containing five sibling-conspecifics and those containing five non-sibling-conspecifics indicating that female *G. ashmeadi* do not recognize siblings as being genetically identical to themselves and do not alter progeny allocation as a result of encountering closely related sibs on host patches. Additionally, pre-exposure to unrelated conspecifics for approximately 19 h had no effect on sex allocation when individual access to host patches occurred; females allocated progeny exactly the same as individual female *G. ashmeadi* not confined with conspecifics. This suggests that prolonged female–female contact immediately prior to locating hosts does not affect future sex allocation and that *G. ashmeadi* modifies sex ratio directly in response to conspecific presence on host egg masses.

4.4. Practical applications of this work

To maximize the percentage female offspring it is recommended that mass rearing facilities producing *Gonatocerus* parasitoids for release in California, and elsewhere, as part of the classical biological control program against *H. coagulata* regulate the number of ovipositing females in cages to maximize the percentage female offspring. This may be beneficial since production of biological control agents is costly and producing excess males wastes resources. The economic significance of inefficient mass rearing is substantial. For mass production of *H. coagulata* parasitoids, total rearing and management costs were estimated at \$606,000 for production of 480,000 parasitoids in 2003 (R. Wynn, GWSS/PDCP Statewide Coordinator, CDFA, pers. comm. 2004; CDFA, 2004). Considering the production of female parasitoids only and using *G. ashmeadi* as an example, at a sex ratio of 89% female (optimal rearing conditions indicated from this study) cost per female parasitoid was estimated at \$1.42 in 2003. Under suboptimal conditions (28% female) cost per female parasitoid increased to \$4.51; a 218% increase in production cost per female. It should also be noted, however, that these calculations do not take into consideration increased costs that may be associated with minimizing female–female contact such as additional rearing cages, increased rearing space and host plants, and additional labor for managing more parasitoid colonies.

Increasing ovipositing female number from 1 to 5 increased percentage male offspring production by up to 61% for all three *Gonatocerus* spp. However, regulation of female densities may be less significant for *G. triguttatus*, which demonstrated a more stable progeny sex ratio in response to increasing female densities. Although percentage male offspring production reached an equilibrium of 42 and 40% for *G. ashmeadi* and *G. triguttatus*, respectively, results indicated that percentage male *G. fasciatus* offspring may continue to increase with increasing numbers of ovipositing females. Therefore, female density regulation will be essential for the gregarious *G. fasciatus*, not only because of increased male production, but also because overcrowding may affect adult fitness because of reduced size as more progeny are produced per host egg.

Mass rearing facilities may attempt to enhance parasitoid production efficiency by stocking oviposition cages with additional females to increase parasitism rates. However, under conditions in the current study this was not achievable for *G. ashmeadi* and *G. fasciatus* as logistic regression analyses indicated that percentage parasitism was not significantly correlated to female density for these two species. Conversely, percentage parasitism by *G. triguttatus* was significantly positively correlated with female density which may indicate that, in order to maximize production efficiency, a compromise needs to be achieved between minimizing the proportion of unparasitized hosts to prevent resource wastage, and maximizing female offspring production.

Overall percentage parasitism by *G. ashmeadi* was significantly higher compared with *G. fasciatus* and *G. triguttatus*, while overall parasitism by *G. fasciatus* was lower compared with *G. ashmeadi* and *G. triguttatus*. These results support earlier observations by Irvin and Hoddle (2005a,b). Clutch size and progeny production data presented in the current study demonstrates the high reproductive capability of *G. fasciatus* because of its gregarious reproductive biology. This trait may be advantageous early in the spring when *H. coagulata* egg masses are relatively rare in California and parasitism by congenics is low (Triapitsyn et al., 2003).

Results suggested that female–female contact prior to locating hosts does not affect future sex allocation and *G. ashmeadi* modifies sex ratio directly in response to conspecific presence on the egg mass, regardless of relatedness. Therefore, it may not be necessary to ensure that female *G. ashmeadi* are collected and caged in low numbers or in sib-groups prior to field releases since production of female offspring and consequent parasitism will not be detrimentally affected. These results confirm that current procedures involving caging 50–200 *G. ashmeadi* per vial before field release are adequate.

The relationship between percentage male progeny production and number of parasitized eggs per egg mass for each *Gonatocerus* spp. studied in this current work may be important for future studies involving collection of egg masses from the field since it could be used determine

whether more than one female parasitized that egg mass, and whether LMC and precise sex allocation is manifested under field conditions where host egg masses are more difficult to locate and physical encounters between female parasitoids are less likely.

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