

# Using hosts of mixed sizes to reduce male-biased sex ratio in the parasitoid wasp, *Diglyphus isaea*

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## Abstract

Overproduction of males in mass rearing of parasitic Hymenoptera contributes to higher costs for biological control because only females directly kill pests. We present a technique, based on manipulating host composition, to generate less male-biased sex ratios in parasitoid species that adjust their sex allocation in response to relative host size. Our system consisted of chrysanthemum, *Dendranthema grandiflora* Tzvelev var. 'Miramar'; a leafminer, *Liriomyza langei* Frick (Diptera: Agromyzidae); and a commercially available parasitoid, *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae). We compared the offspring sex ratios of *D. isaea* females presented with different compositions of *L. langei* larvae on chrysanthemum. Presenting individual females with only large hosts increased mean sex ratio from 32 to 67% male over 2 days. However, presenting individual females with progressively larger hosts over 1 or 2 days reduced mean sex ratio from 90 to 100% male to less than 30% male. Groups of females produced sex ratios around 58% male if presented with both plants infested by only small hosts and plants infested by only large hosts. In comparison, groups of females produced sex ratios around 48% male if presented with plants infested by both small hosts and large hosts. We compared the use of both small hosts and large hosts to only large hosts for simulated mass rearing of wasps over 8 weeks. Using both small hosts and large hosts produced similar numbers of wasps as using only large hosts, but reduced mean sex ratio of weekly cohorts from 66% male to 56% male. The two techniques produced females of similar size, but using both large hosts and small hosts produced slightly smaller males than using only large hosts. The use of both small hosts and large hosts for mass rearing of *D. isaea* could reduce actual costs of females by 23%.

## Introduction

A common problem with augmentative biological control is the high cost for the large numbers of control agents needed for suppressing pests at the early stages of infestation. In mass rearing of parasitic Hymenoptera, overproduction of males contributes to the costs of production and control. Only female parasitoids kill hosts directly by oviposition or host feeding or both. Thus, excess males (more than needed to mate with available females) can increase the cost for establishing and then maintaining pest control.

An example is *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae), a commercially available parasitoid recommended for the control of *Liriomyza* leafminers on greenhouse vegetables (reviewed in van der Linden, 2004) or flower crops that tolerate damage early in the production

cycle (reviewed in Chow & Heinz, 2004). *Diglyphus isaea* is a solitary larval ectoparasitoid; females paralyze their host with venom and oviposit on or near larger hosts but reject or host feed on smaller hosts (Ode & Heinz, 2002). Partly because of male-biased sex ratios in commercial shipments (60–70% male as reported in Heimpel & Lundgren, 2000), control of *Liriomyza* leafminers with *D. isaea* is more costly than using insecticides.

Successful control of *Liriomyza* leafminers with *D. isaea* on cut-flower chrysanthemums in greenhouses may require weekly releases of up to 400 wasps (60–70% male) per 1000 plants during the entire 11-week crop cycle (Parrella et al., 1992; Heinz et al., 1993). Control of *Liriomyza* leafminers on 1000 chrysanthemums with insecticides would cost around US\$600 (M Smith, pers. comm.) compared to almost US\$1700 with *D. isaea* (current pricing, Koppert USA, Romulus, MI, USA). Presently, U.S. clientele pays an actual cost of up to US\$1.00 per female wasp for *D. isaea*.

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Manipulating behavior of *D. isaea* to produce less male-biased sex ratios may increase rearing efficiency and reduce prices.

Manipulation of offspring sex ratio in parasitoid wasps is predicted by theory and supported by empirical studies (reviewed in King, 1993; Godfray, 1994). Mated females determine offspring sex by controlling fertilization because fertilized (diploid) eggs develop as females and unfertilized (haploid) eggs as males (Flanders, 1956). Host quality influences sex allocation in many species of parasitoids (Charnov, 1982; Hardy, 1994). When host quality (often measured as size) differentially affects progeny fitness, selection will favor mothers that manipulate sex ratio in response to current host quality distributions (Bull, 1981; Charnov et al., 1981).

The host-quality model of Charnov et al. (1981) applies to solitary parasitoids where adult size is closely correlated with host size. Charnov et al. (1981) argued that if host size affects female fitness more than male fitness, female wasps should allocate daughters to larger hosts and sons to smaller hosts. Moreover, Charnov's host-quality model also predicted that relative, rather than absolute, host size is important. In other words, a female wasp will assess a host to be large or small depending upon the distribution of host sizes encountered. Thus, females eventually produce similar sex ratios if they encounter only small or only large hosts.

Tests of these predictions with *D. isaea* (Ode & Heinz, 2002) generally support Charnov's host-quality model. Laboratory sex ratios of *D. isaea* tend to be male-biased, but host size positively affects fitness in both sexes, and females lay more daughters on large hosts and more sons on smaller hosts. Female *D. isaea* also seem to assess host size on a relative basis. Providing groups of females with increasingly larger hosts on each of three successive days will reduce sex ratios from 64% to 45% male. Females apparently update their perception of host-size distributions by comparing the sizes of hosts from present and past encounters.

Although increasing host size over time can reduce overproduction of males in *D. isaea*, there are problems with this technique for mass rearing. First, producing cohorts of different-sized hosts requires extra labor, host plants, and space. Second, a limited range of host sizes will constrain the length of time over which parasitoid behavior can be manipulated. As an alternative technique, we propose presenting *D. isaea* females with hosts of mixed sizes simultaneously.

Host composition and distribution will affect the relative encounter rate of female *D. isaea* with different-sized hosts and thus influence sex allocation. Even females presented with a population of only large hosts may eventually produce male-biased sex ratios because their perception of relative host sizes (large vs. small) will change with experience.

However, females presented with an equal mixture of large hosts and much smaller hosts will frequently encounter both host types. Distribution of the two host types in the environment will influence relative encounter rates, but females encountering mixed host populations should almost always lay daughters on large hosts and only enough sons on small hosts to mate with all their daughters.

In this paper, we evaluated manipulation of host composition and distribution to reduce male-biased sex ratios in *D. isaea*. We used *Liriomyza langei* Frick (Diptera: Agromyzidae) as the host because this species is larger as a larva than most other leafminers commonly attacked by *D. isaea* (Spencer, 1989). By using *L. langei*, we maximized size differences between large hosts and small hosts for our experiments.

## Materials and methods

### Insect colonies and rearing procedures

Colonies of *L. langei* were established from pupae collected near Salinas, CA, USA. Colonies of *D. isaea* were started from adult wasps obtained from Koppert USA. We reared *L. langei* on chrysanthemum (*Dendranthema grandiflora* Tzvelev var. 'Miramar') and *D. isaea* on *L. langei* following the protocol described by Ode & Heinz (2002). Plants were grown from cuttings for at least 1 month in greenhouses. Development from egg to adult for *L. langei* was around 20–22 days and 15–17 days for *D. isaea* at 20–25 °C and L14:D10.

To obtain uniform cohorts of *L. langei* larvae, we exposed chrysanthemums to adult leafminers for about 4 h in cages and then held the plants at 20–25 °C. We categorized 8-day-old larvae as large hosts, 7-day-old larvae as intermediate-sized hosts, and 6-day-old larvae as small hosts. Large hosts were approximately 3 to 4 times the size of small hosts. The area (length × width) of these hosts ranged from 0.45 to 1.10 mm<sup>2</sup> (small), 0.84 to 1.86 mm<sup>2</sup> (intermediate), and 2.04 to 3.51 mm<sup>2</sup> (large).

To standardize wasps for experiments, we selected wasps of similar size and age for each sex from cohorts that developed on 8–9-day-old *L. langei*. Equal numbers (10–15) of adult males and adult females (1–2 days old) were confined together in paper cups (9.5 cm diameter × 5.5 cm height) with honey solution for at least 48 h before experiments. We reared insect colonies and conducted experiments at 20–25 °C, 40–70% r.h., and L14:D10. All colonies were laboratory reared for at least 2 months before being used for experiments.

### Experiment 1. Host size variation and sex allocation

Our ability to reduce male-biased sex ratio in *D. isaea* depends on how fast individual females adjust sex allocation

in response to variation in host size. If female wasps adjust sex allocation quickly, it will favor the use of mixed host sizes to reduce overproduction of sons. We preconditioned wasps for this study because *D. isaea* females require host feeding to produce large numbers of eggs (Minkenberg, 1989). Groups of three female wasps (3–4 days old) were confined for 24–48 h in mesh-screened Petri dish cages (15.5 cm diameter  $\times$  4.0 cm height) with chrysanthemum leaves infested with 8–14 intermediate-sized hosts. By preconditioning *D. isaea* on intermediate-sized hosts, we also provided female wasps with a ‘gauge’ for assessing host size in subsequent treatments.

Each preconditioned female was confined in a Petri dish cage with an infested leaf and given one of three treatments: stable host size over 2 days, increasing host size over 2 days, or increasing host size over 1 day. Females given the ‘increasing host size over 1 day’ treatment were exposed to small hosts for 4 h and then large hosts in another cage for 20 h. In comparison, females given the ‘increasing host size over 2 days’ treatment were exposed to small hosts on day 1 and large hosts on day 2. Females given the ‘stable host size’ treatment were exposed to large hosts on day 1 and a different set of large hosts on day 2.

We screened all treated females to minimize sex ratio variation due to mating status or sperm quality. After a treatment, each female was confined with large hosts for 48 h. Only females that produced daughters on these large hosts were counted as treatment replicates.

We measured the hind tibia length of all females counted as replicates and completed 12 replicates for each of the three treatments (total = 36 wasps). All live or dead leafminers in each cage were counted within 3–4 days after each treatment. Wasp progeny that developed in each cage were sexed and counted.

#### **Experiment 2. Effect of host composition and distribution on sex allocation**

We tested groups of *D. isaea* on different combinations and distributions of large and small hosts on potted plants to assess the degree to which sex allocation of mass-reared populations could be altered. To simulate mass rearing, we did not precondition female wasps on hosts and included male wasps. Groups of 40 female wasps and 40 male wasps (4–5 days old) were confined in mesh-screened cages (46  $\times$  38  $\times$  51 cm) and exposed for 48 h to one of three treatments: four pots of plants infested with only large hosts (Lg), two pots of plants infested with only small hosts and two pots of plants infested with only large hosts (Lg or Sm), or four pots of plants infested with similar numbers of both small hosts and large hosts (Lg + Sm). Each pot contained three plants for a total of 12 plants per treatment.

To obtain plants with both small hosts and large hosts, we caged nine plants for 1.5–2 h with adult leafminers and repeated the procedure 2 days later. We caged nine plants for 3–4 h with adult leafminers to obtain plants with only large hosts. When leafminer larvae were of the required ages, we selected plants of uniform height (12–18 cm), leaf number (7–12 mature leaves), and host density (16–28 leafminer larvae) for treatments.

Four replications were completed for each of the three treatments (one replication = 40 female wasps + 40 male wasps, total = 960 wasps). We counted all the leaves, wasp pupae, and dead hosts on each plant about 13–14 days after each treatment. The leaves of each plant were then cut and confined in Petri dishes. Adult wasps that enclosed in the dishes were sexed and counted.

#### **Experiment 3. Sex ratios under simulated mass rearing**

We evaluated two host-presentation techniques, only large hosts or both large hosts and small hosts, for simulated mass rearing of *D. isaea*. To simulate mass rearing, we did not precondition female wasps on hosts. Two groups of 80 female wasps and 80 male wasps (4–5 days old) were each confined in a different mesh-screened cage. For three consecutive days, each group was exposed to nine plants infested with immature leafminers. Every 24 h, the plants were replaced with another set of identically infested plants. One group was exposed to plants infested with only large hosts (Lg). The second group was exposed to plants infested with similar numbers of both large hosts and small hosts (Lg + Sm).

We maintained 160–200 wasps in each cage by adding 20 female wasps and 20 male wasps whenever plants were changed. This protocol was repeated for 8 consecutive weeks to produce 24 wasp cohorts for each technique. Colony plants were cut when immature wasps were approximately 13 days old. Cut plants containing wasp cohorts produced by the same technique during the same week were held together. Adult wasps that enclosed from each weekly harvest were sexed and counted. To compare the size of wasps produced by the two techniques, we measured the hind tibia length of 40 female wasps and 40 male wasps from the second, fourth, sixth, and eighth harvests of each technique.

#### **Statistical analyses**

Sex ratio was the proportion of males among offspring. Wasp mortality was the proportion of immature *D. isaea* that did not become adults. Host mortality was the proportion of immature leafminers killed or parasitized. For ANOVA, we transformed numbers of offspring by individual females to their square-root values and both sex ratio and wasp mortality to their arcsine values. Back-transformed means are reported with 95% confidence

**Table 1** Mean sex ratio (proportion of males) and numbers of offspring produced by individual females of *Diglyphus isaea* in host-presentation regimes (Experiment 1) including 6-day-old (Sm) and 8-day-old (Lg) *Liriomyza langei* reared inside chrysanthemum leaves at 20–25 °C and L14:D10. Each female ( $n = 12$  for each regime) was individually confined for either 24, 20, or 4 h in a mesh-screened cage containing a single leaf infested with only one type of host

Host presentation regime <sup>a</sup>	Exposure intervals	No. of hosts <sup>b,c</sup> Mean (95% CI)	No. of offspring <sup>b,c</sup> Mean (95% CI)	Sex ratio <sup>b,d</sup> Mean (95% CI)
24 h Lg : 24 h Lg	1st (24 h Lg)	11.92 (10.59–13.32) <sup>ns</sup>	5.38 (3.25–7.98) <sup>ns</sup>	0.32 (0.15–0.52)*
	2nd (24 h Lg)	11.34 (9.21–13.69)	7.00 (4.85–9.52)	0.67 (0.41–0.89)
24 h Sm : 24 h Lg	1st (24 h Sm)	9.02 (8.02–10.08) <sup>ns</sup>	6.79 (4.74–9.16)*	0.98 (0.93–1.00)**
	2nd (24 h Lg)	11.72 (9.37–14.31)	9.97 (7.83–12.36)	0.17 (0.08–0.29)
4 h Sm : 20 h Lg	1st (4 h Sm)	9.55 (7.75–11.52) <sup>ns</sup>	2.63 (1.67–3.78)**	0.99 (0.95–1.00)**
	2nd (20 h Lg)	9.10 (8.03–10.23)	6.29 (4.32–8.59)	0.29 (0.14–0.45)

<sup>a</sup>Females given the 24 h Lg : 24 h Lg treatment were exposed to large hosts for 24 h and then transferred to another cage and exposed to a different set of large hosts for 24 h. Females given the 24 h Sm : 24 h Lg treatment were exposed to small hosts for 24 h and then transferred and exposed to large hosts for 24 h. Females given the 4 h Sm : 20 h Lg treatment were exposed to small hosts for 4 h and then transferred and exposed to large hosts for 20 h.

<sup>b</sup>Differences between the numbers of hosts, numbers of offspring, or sex ratios from the first and second exposure intervals of each presentation regime were tested by the Wilcoxon match-pairs signed-rank test for statistical significance (<sup>ns</sup>  $P \geq 0.05$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ ).

<sup>c</sup>Numbers of hosts and offspring were transformed to their square-root values for ANOVA. Means and confidence intervals were obtained by back-transformation of the square-root values.

<sup>d</sup>Sex ratios were transformed to their arcsine values for ANOVA. Means and confidence intervals were obtained by back-transformation of the arcsine values.

intervals. One-way ANOVA was used to compare between treatments: size of female wasps used, number of leaves, number of hosts, host mortality, wasp mortality, number of offspring, and sex ratio. We used the Wilcoxon matched-pairs signed-rank test to compare numbers of hosts in treatments for individual females, sex ratios and numbers of progeny produced by individual females, and sex ratios and numbers of wasps from weekly harvests. Two-level nested ANOVA was used to compare the size of female wasps or male wasps produced by the two host-presentation techniques. Three-level nested ANOVA was used to compare the size of female wasps and male wasps from the same weekly harvests.

## Results

### Experiment 1. Host size variation and sex allocation

Female wasps used in the three treatments were similar in size (hind tibia length: mean  $\pm$  SEM =  $0.48 \pm 0.01$  mm;  $F_{2,33} = 0.015$ ,  $P = 0.985$ ). Individual females exposed to increasing or stable host size distributions produced substantially different offspring sex ratios during the first ( $F_{2,33} = 42.415$ ,  $P \leq 0.001$ ) and second ( $F_{2,33} = 9.389$ ,  $P \leq 0.001$ ) exposure intervals (Table 1). On day 1, females exposed to only large hosts produced around 32% male offspring, whereas females exposed to only small hosts produced 93–100% male offspring. However, we could not maintain female-biased sex ratios with only large hosts. Females exposed to only large hosts produced around 67% male offspring

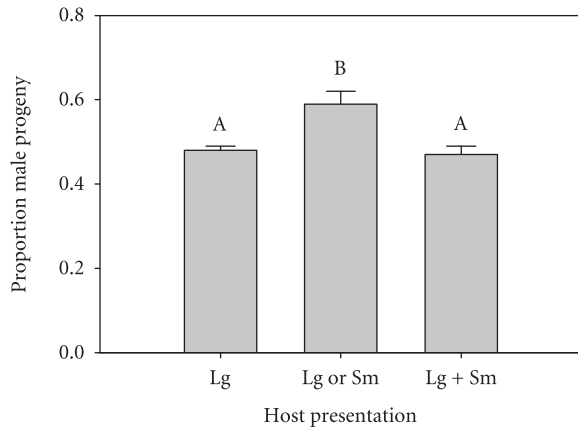
on day 2. In comparison, females exposed to increasingly larger hosts over 1 or 2 days produced 3 to 5 times fewer sons on large hosts than small hosts (Table 1).

Females produced similar numbers of offspring when exposed to small hosts or large hosts for 24 h (Table 1). The numbers of available hosts were not statistically different among treatments during the second exposure interval (mean = 10.69, 95% CI = 9.62–11.82;  $F_{2,33} = 2.582$ ,  $P = 0.091$ ) or between the first and second exposure intervals of treatments (Table 1). However, there were slightly more hosts (10–14 per cage) in cages with large hosts than small hosts during the first exposure interval (7–11 per cage) ( $F_{2,33} = 42.415$ ,  $P = 0.012$ ).

### Experiment 2. Effect of host composition and distribution on sex allocation

Host composition and distribution had a highly significant effect on the offspring sex ratios produced by groups of *D. isaea* ( $F_{2,9} = 11.704$ ,  $P = 0.003$ ). Wasps exposed to only large hosts (Lg) or both small hosts and large hosts on the same plants (Lg + Sm) produced sex ratios around 48% male (Figure 1). However, wasps exposed to small hosts on half the plants and large hosts on the other plants (Lg or Sm) produced approximately 10% more males than the other two groups (Figure 1).

Female wasps produced similar numbers of offspring in all treatments (mean  $\pm$  SEM =  $221.6 \pm 31.0$ ;  $F_{2,9} = 0.631$ ,  $P = 0.613$ ). Counts of adult wasps were fairly accurate



**Figure 1** Offspring sex ratios produced by groups of 40 *Diglyphus isaea* females and 40 males exposed for 48 h to one of three different host-size treatments: potted chrysanthemum infested with 8-day-old *Liriomyza langei* (Lg), potted chrysanthemum infested with only 8-day-old *L. langei* or only 6-day-old *L. langei* (Lg or Sm), or potted chrysanthemum infested with both 8-day-old and 6-day-old *L. langei* (Lg + Sm). Four different groups of wasps were exposed to each treatment. Columns showing the same letter are not significantly different ( $P \geq 0.05$ ) by pairwise comparisons of means using the Bonferroni method (Sokal & Rohlf, 2000). Sex ratios were transformed to their arcsine values for analysis, but untransformed means (+ SE) are shown in this graph.

representations of sex allocation by *D. isaea* because 99% of wasps, on average, successfully developed into adults. Wasp mortality was uniformly low among all treatments (mean = 0.009, 95% CI = 0.004–0.015;  $F_{2,9} = 0.124$ ,  $P = 0.885$ ). Host densities in the three treatments were comparable because the numbers of leaves (mean  $\pm$  SEM =  $113.6 \pm 1.6$ ;  $F_{2,9} = 1.247$ ,  $P = 0.333$ ) and hosts (mean  $\pm$  SEM =  $477.0 \pm 48.9$ ;  $F_{2,9} = 0.303$ ,  $P = 0.746$ ) per cage did not vary significantly among treatments. Host composition and distribution did not seem to affect the foraging success of female wasps because host mortality did not vary significantly among treatments (mean  $\pm$  SEM =  $0.56 \pm 0.05$ ;  $F_{2,9} = 1.251$ ,  $P = 0.331$ ).

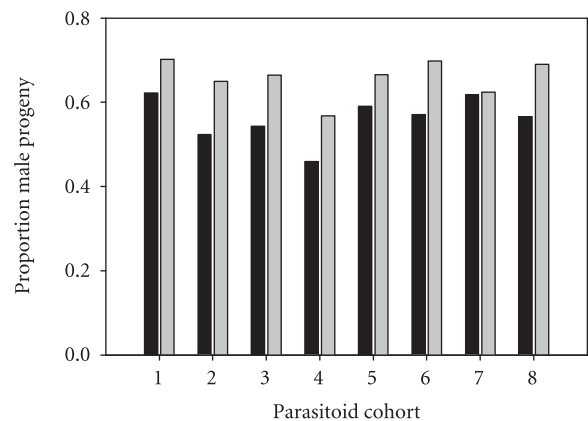
### Experiment 3. Sex ratios under simulated mass rearing

The two host-presentation techniques produced similar numbers of wasps over the eight weekly harvests (mean  $\pm$  SEM =  $560.25 \pm 40.08$ ;  $n = 16$ ) ( $n = 8$ ;  $Z = -0.980$ ,  $P = 0.327$ ), but substantially different offspring sex ratio ( $n = 8$ ;  $Z = -2.521$ ,  $P = 0.012$ ). The 'Lg + Sm' technique always produced weekly harvests with fewer males (1–13% less) than the 'Lg' technique (Figure 2). Mean sex ratio for harvests produced by the 'Lg + Sm' technique (mean  $\pm$  SEM =  $0.56 \pm 0.02$ ;  $n = 8$ ) was 10% less male biased than for the 'Lg' technique (mean  $\pm$  SEM =  $0.66 \pm 0.02$ ;  $n = 8$ ).

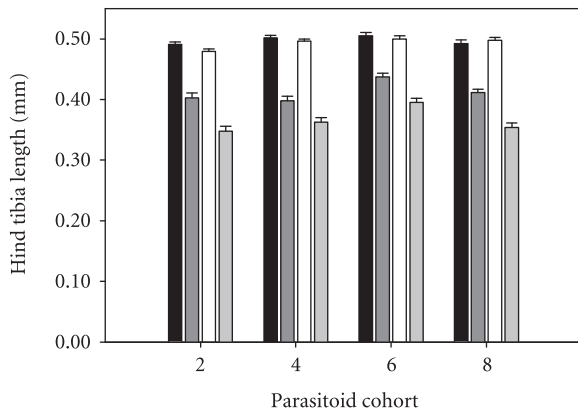
Both techniques produced female wasps of similar size ( $F_{1,6} = 0.491$ ,  $P = 0.510$ ), but the 'Lg + Sm' technique consistently produced smaller males than the 'Lg' technique ( $F_{1,6} = 11.722$ ,  $P = 0.014$ ) (Figure 3). Females were always larger than males from the same harvests ( $F_{2,12} = 104.685$ ,  $P \leq 0.001$ ) and wasp size varied slightly among weekly harvests (Figure 3).

## Discussion

Field collections for *Diglyphus begini* (Ashmead) (60–70% male) (Heinz & Parrella, 1990) and *Diglyphus intermedius* (Girault) (68% male) (Coote & Ellis, 1986) suggest that male-biased sex ratios are common in *Diglyphus* species. The literature does not report any field sex ratios for *D. isaea*; however, sex ratios produced by the 'Lg' mass-rearing technique (around 67% male) are similar to field sex ratios for other *Diglyphus* species. Host composition and distribution can influence laboratory sex ratios of *D. isaea*, but little is known about the ecological constraints that affect sex allocation of this wasp under natural conditions. *Diglyphus isaea* and its congeners probably encounter many host types and host distributions in the field. Salvo & Valladares (2002) found that the size of *Liriomyza* leafminers and their suitability for parasitoids vary with the plant host in both natural habitats and cultivated areas. Spatial distributions of *Liriomyza* larvae range from clumped to random on different crops (Heinz & Chaney, 1995; Abou-Fakhr Hammad & Nemer, 2000). Female wasps may optimize reproduction in a varying environment by adjusting sex allocation for long-term or average returns in fitness



**Figure 2** Offspring sex ratios of parasitoid cohorts harvested weekly over 8 consecutive weeks from two colonies of *Diglyphus isaea*. Parasitoids in one colony were exposed only to potted chrysanthemums infested with 8-day-old *Liriomyza langei* (Lg) □. Parasitoids in the other colony were exposed to potted chrysanthemums infested with approximately equal numbers of both 6-day-old and 8-day-old *L. langei* (Lg + Sm) ■.



**Figure 3** Hind tibia lengths (mm) of males and females of *Diglyphus isaea* randomly selected from four of eight groups of wasps harvested weekly over 8 consecutive weeks from two different colonies. Parasitoids in one colony were exposed only to potted chrysanthemums infested with 8-day-old *Liriomyza langei* (females = ■; males = ▒). Parasitoids in the other colony were exposed to potted chrysanthemums infested with approximately equal numbers of both 6-day-old and 8-day-old *L. langei* (females = □; males = ▒). Each column represents the mean (+ SE) for 40 individual wasps.

(Cohen, 1966). Determining whether male-biased sex ratios are optimal for *D. isaea* will require studies on host-size distributions, host densities, mating success, and sex ratios for this wasp in nature.

Host-size-dependent sex allocation is widespread in solitary idiobionts (King, 1987, 1989, 1993), including many commercially available species (Hunter, 1997). Interestingly, not all parasitoid species showing host-size advantage effects can adjust offspring sex ratios in response to changes in the relative abundance of different-sized hosts (Jones, 1982). For example, females of encyrtid wasps such as *Epidinocarsis lopezi* (De Santis) (van Dijken et al., 1991) and *Metaphycus stanelyi* Compere (Bernal et al., 1998) lay primarily males in small hosts regardless of the relative proportion of small to large hosts. Our method for reducing male-biased sex ratios has the highest probability of success with solitary parasitoids exhibiting sex allocation behavior similar to *D. isaea*.

Female *D. isaea* appears to quickly adjust sex allocation in response to variation in host size. To minimize male production, Ode & Heinz (2002) presented small, intermediate, and then large larvae of *Liriomyza huidobrensis* (Blanchard) over 3 days to individual or groups of *D. isaea* females. Heinz (1998) also exposed groups of *Catolaccus grandis* (Burks), a boll-weevil parasitoid, to increasing host sizes over 4 days to reduce male-biased sex ratios. We shifted offspring sex ratio from around 99% male to less than 30% male by exposing individual female *D. isaea* to small and then large hosts over 1 day. By presenting female wasps

with plants infested with both large hosts and small hosts, we can manipulate their quick response to host size variation without progressively increasing host size over time.

Ode & Heinz (2002) found that individual female *D. isaea* produces sex ratios of around 60% male if exposed to uniform host sizes over 3 days. We found that presenting individual females with only large hosts produced sex ratios around 32% male on day 1 and around 67% male on day 2. However, our females were preconditioned on intermediate-sized hosts before treatments. Using a memory model, Ode & Heinz (2002) showed how female *D. isaea* are influenced more by recent than past host encounters in their assessment of host size for sex allocation. Without encounters with smaller hosts, females eventually produce male-biased sex ratios on even large hosts.

In our study, female *D. isaea* produced more male-biased sex ratios when presented with both small hosts and large hosts on separate plants as compared to both host types on the same plants. Cheah & Coaker (1992) found that female *D. isaea* systematically and repetitively search and probe chrysanthemum foliage infested by *Chromatomyia syngenesiae* Hardy. Systematic searching may be the most successful strategy for parasitoids of hosts that do not move extensively or are confined within a patch. Having both small hosts and large hosts on the same plant may increase the frequency of encounters with both host types and promote the use of large hosts for daughters and smaller hosts for sons.

We found that presentation of both large hosts and small hosts to *D. isaea* produced smaller male wasps than presentation of only large hosts. In *D. begini*, reproductive success of small males is lower than that of larger males at high wasp densities (Heinz, 1991). To assess possible differences in the quality of *D. isaea* due to rearing technique, we evaluated wasps in greenhouse trials simulating commercial production of chrysanthemum. We compared the efficacy of *D. isaea*, produced by our two rearing techniques, for preventing crop damage by *L. langei* and found no significant differences (A Chow, unpubl.).

In summary, we used both small hosts and large hosts to reduce male-biased sex ratios in *D. isaea*. Manipulation of parasitoid behavior to reduce overproduction of males can increase mass-rearing efficiency and reduce prices for clientele. Even if the cost per wasp shipment did not change, clientele will benefit because fewer shipments would be needed for pest suppression. For example, a single order of *D. isaea* costs US\$75.00 and contains about 250 wasps, but only 85 would be female assuming a sex ratio of 0.66 (typical for commercial shipments). However, shipments with a sex ratio of 0.56 (attainable with our rearing technique) would have 110 females; therefore, the effective cost per female wasp would decrease from US\$0.88 to US\$0.68. With all else being equal and further refinement, our rearing

technique could help bring the cost of leafminer biological control more in line with pesticide-based programs.

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