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Host-size-dependent sex ratio theory and improving mass-reared parasitoid sex ratios

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Abstract

Although an effective parasitoid of agromyzid leafminers, *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) is an expensive biological control agent in terms of production costs. In part, these costs arise from the production of male-biased offspring sex ratios. Here, we present a mass-rearing technique that will increase the proportion of females produced and reduce the need for frequent releases in biocontrol programs. By presenting female *D. isaea* groups of sequentially larger leafminer hosts to attack, we are able to generate progressively more female-biased sex ratios. After three days of providing increasingly larger hosts, we were able to reduce the sex ratios produced by individual females from 57% male to 36% male; sex ratios produced by groups of females dropped from 64% male to 45% male. Several attributes of *D. isaea* sex allocation allow us to manipulate sex allocation behavior. First, *D. isaea* is a solitary idiobiont; resources available to each offspring are present at the time of attack allowing the ovipositing female to accurately assess host quality. Host size positively affects both male and female wasps. Females laid more daughters in larger hosts and more sons in smaller hosts. We show that the observed relationship between host size and offspring sex ratio is due to maternal sex allocation decisions rather than differential mortality. Furthermore, assessment of the size threshold was relative to prior host encounters rather than an absolute assessment. Our simple memory model suggests that while females are influenced most strongly by recent encounters, females also base their assessment of the host-size threshold on prior host encounters. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: *Liriomyza huidobrensis*; Serpentine leafminers; *Diglyphus isaea*; Host-size dependent sex ratio theory; Augmentative biological control

1. Introduction

In many agricultural and urban landscapes tolerance to arthropod damage is very low. One solution for pest control in these situations is the repeated release of natural enemies through augmentative or inundative biological control. While these approaches can provide desirable levels of pest suppression (van Lenteren, 1986; van Lenteren et al., 1997), they are often prohibitively expensive relative to chemical control programs in terms of production and implementation costs (E.G. King, 1993; Heinz, 1998; Parrella et al., 1992). As a result, many otherwise very effective biological control agents are not reared by commercial insectaries.

Modification of mass-rearing procedures to reduce the production costs of natural enemies is one strategy that could make available otherwise unaffordable natural enemies for use in biological control. If purchase costs are substantially reduced, biological control is more likely to be perceived as a viable alternative to traditional chemical control.

In augmentative releases, females are more valuable than males because only they are directly responsible for killing pests by oviposition and/or host feeding. One important cost associated with many arrhenotokous parasitic Hymenoptera (parasitoid) mass-rearing programs is the production of more males than is necessary to mate females that are produced. Excess males represent a surplus that can make the commercial production costs of parasitoids excessive in terms of the cost-per-female-released. Mass-rearing programs that incorporate techniques to increase the proportion of females produced may permit insectaries to produce a higher

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quality product with little or no increase in production costs. While the price per natural enemy shipment charged to clientele may not change, biocontrol practitioners may realize significant savings if they are able to attain the same degree of pest control with fewer natural enemy releases.

Here we present a technique, based on manipulating host size, to generate more female-biased sex ratios in the eulophid wasp *Diglyphus isaea* Walker. *D. isaea* is a solitary, larval ectoparasitoid of agromyzid leafminers including the pea leafminer, *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae), the host used in this study. *D. isaea* and its congener, *Diglyphus begini* (Ashmead), are effective augmentative biological control agents against *Liriomyza* leafminers infesting a wide range of greenhouse and field-grown crops and ornamentals (del-Bene, 1990, 1994; Gordh and Hendrickson, 1979; Heinz et al., 1993; Heinz and Parrella, 1990a; Jones et al., 1986; McClanahan, 1977; Minkenberg, 1989; Wardlow, 1985). Large-scale mass-production programs for *D. isaea* (Hendrickson, 1975; Hunter, 1997) and its host (Heinz, 1996; Parrella et al., 1989) have been developed to support biological control efforts using this species. However, current rearing procedures for *D. isaea* remain expensive (van Lenteren, 1986), in part because of the production of male-biased sex ratios (0.6–0.7 proportion male; Hendrickson and Barth, 1978; Ode and Heinz, unpublished data). As a consequence, adoption of this species as a biocontrol agent is hindered (Daniel Cahn, Syngenta Bioline, personal communication). Commercial prices charged per individual wasp range from \$0.065 to \$0.349 making this a relatively expensive biocontrol agent compared to other mass-reared parasitoids (van Lenteren et al., 1997). Costs of biocontrol using *Diglyphus* spp. are compounded by the need for frequent, large releases to achieve acceptable levels of leafminer control (Heinz et al., 1988). Development of a rearing system that maximizes female wasp production may reduce overall mass-rearing costs and, thereby, increase the attractiveness of using *D. isaea* to control agromyzid leafminers in commercial settings.

Liriomyza huidobrensis is an economically important pest of a wide range of greenhouse and field-grown plants. Female flies lay eggs by first puncturing the upper surface of the leaf and depositing an egg in the tubular leaf puncture. Adult females feed from the oviposition site as well as from other leaf punctures (Parrella, 1987). Leaf puncturing by feeding adults and mine construction by feeding larvae result in serious aesthetic damage to ornamental plants and certain crop plants such as celery (Heinz and Chaney, 1995). Both feeding activities substantially reduce photosynthetic capacity resulting in significant yield reductions (Parrella et al., 1985; Rauf et al., 2000; Shepard et al., 1998; Weintraub and Horowitz, 1996).

Once a *D. isaea* female encounters a host, she antennates the mine directly over the leafminer larva. Before oviposition, *D. isaea* females inject paralytic venom into the host. Therefore, the size of the host at oviposition represents the amount of resource available to the developing offspring. Like its well-studied congener *D. begini*, *D. isaea* females oviposit on larger hosts but reject or host feed on smaller hosts (Heinz and Parrella, 1989, 1990a).

Host quality is well known to influence the sex allocation decisions made by many species of parasitic wasps (Charnov, 1982; Godfray, 1994; King, 1987). When host quality, most frequently measured in terms of size, differentially affects the fitness of sons and daughters, selection will favor mothers with the ability to manipulate sex ratio in response to current host quality distributions (Bull, 1981; Charnov, 1979; Charnov et al., 1981). Researchers have shown repeatedly that many parasitoids lay male eggs in smaller hosts and female eggs in larger hosts (Clausen, 1939; Charnov, 1982; Godfray, 1994; King, 1987). Charnov et al. (1981) were the first to explain how this common sex allocation pattern in parasitoids may be adaptive. Their host quality model was developed specifically for solitary species where at most one adult offspring develops per host and the quantity of resources available to a developing wasp is expected to correlate closely with the size of the host (Godfray, 1994; B.H. King, 1987, 1993). In many species of parasitoids, adult wasp size is tightly correlated with the size of the host in which it developed. Charnov et al. (1981) argued that host size affects female fitness more strongly than male fitness; therefore, an ovipositing mother will realize greater reproductive success by allocating daughters to larger hosts and sons to smaller hosts. The handful of studies that compare male and female reproductive success, including the one on *D. begini* (Heinz, 1991), have generally supported the assumption that female reproductive success is more positively affected as host size increases (Charnov et al., 1981; Jones, 1982; Ode et al., 1996; Ueno, 1999; van den Assem et al., 1989; but see King, 1988).

Charnov's host quality model makes two additional predictions relevant to this study. First, a critical size threshold exists below which only males are laid and above which only females are laid. Second, relative, rather than absolute, host size is important. For instance, if a female wasp encounters a population of hosts that are small-sized and medium-sized, she should lay more sons in the small-sized hosts and more daughters in the medium-sized hosts. If that same female were to encounter subsequently a population of medium-sized and large-sized hosts, she should now lay sons in the medium-sized hosts and daughters in the large hosts. This prediction has received support in the few studies that have empirically examined this issue (e.g., Charnov et al., 1981; Heinz and Parrella, 1989, 1990b;

Ode and Strand, 1995). Yet, not all species exhibiting host-size advantage effects appear to be capable of within-generation shifts in sex allocation (e.g., Bernal et al., 1998; Jones, 1982; van Dijken et al., 1991).

The predictions of the host-size model are based on the presumption that the ovipositing female has perfect knowledge of the size distribution of the hosts available to her. Yet, ovipositing females have incomplete information regarding the actual distribution of host sizes in the environment. Our ability to modify mass-reared sex ratios depends on the notion that foraging females update their perceptions of host-size distributions in the environment by comparing the sizes of current host encounters with those of past host encounters. Because recent information is likely to be more informative of current conditions in a changing environment (Bernstein et al., 1988, 1991; Cowie, 1977; Devenport and Devenport, 1994; Iwasa et al., 1981; Ollason, 1980; Persons and Uetz, 1998; Roche et al., 1998), assessment of what are large and small hosts is likely to be based on a combination of past and current foraging experiences with recent experiences receiving more weight. A female's perception of the population average host size will be updated with every new host encounter. If the environment changes slowly, then information gathered remains useful for a longer period of time. On the other hand, if the environment changes rapidly, previously gathered information has a much shorter period of usefulness (McNamara and Houston, 1980).

We propose to induce more female-biased mass-reared sex ratios by presenting females with sets of increasingly larger hosts faster than they are able to re-adjust their perception of the population average host size. We first examine the relationship between host size and body size of male and female wasps. Second, we present evidence that sons are allocated to small hosts and daughters to large hosts and that this allocation decision is based on a relative rather than an absolute assessment of host size. To do this, we sampled our *D. isaea* colony biweekly and examined the size of the hosts used for males and females. We also examined the sex allocation decisions made by individual females with respect to host size. Third, the relationship between host size and progeny sex ratio may arise from the sex allocation behavior of the mother or it may be the result of differential mortality of male and female offspring. We distinguish these two possibilities by experimentally transferring eggs from both small and large hosts onto large hosts. Fourth, we attempt to induce individual ovipositing females to lay increasingly female-biased sex ratios by presenting them increasingly larger hosts over a three-day period. We repeat this approach with groups of 20–30 females to simulate conditions that might occur in a mass-rearing situation. Finally, we develop a temporal weighting rule model that weights the influence of a given host encounter based on its recency. We deter-

mine empirically the weighting parameter that appears to be operating in *D. isaea*, indicating the degree to which we can expect sex allocation to be biased.

2. Materials and methods

2.1. Rearing procedures

The *L. huidobrensis* colony was established from approximately 2000 pupae field-collected in September 1998 from lettuce near Salinas, California. Adult flies were held in two 50 cm *l* × 50 cm *w* × 50 cm *h* clear acrylic cages. Five pots containing three-week-old chrysanthemum plants grown from rooted cuttings (*Dendranthema grandiflora* Tzvelev var. 'Miramar' and var. 'Charm') were placed in each cage for 24 h. Plants were then removed and held for nine days. Cages and plants were located in a room with a 16:8 (L:D) h photoperiod at 25 °C. Half of the plants containing third instar *L. huidobrensis* were then cut and placed on a wire mesh covering a sand-filled plastic tray (45 × 36 cm). As the larvae finished feeding, they exited the leaf tissue and dropped into the sand where they pupated. Pupae were collected from the sand and were allowed to emerge in the oviposition cages. At any given time, between 500 and 1000 adult flies were present in each oviposition cage.

The remaining half of the plants containing third instar leafminers were placed inside of two similar sized (50 cm *l* × 50 cm *w* × 50 cm *h*) acrylic cages containing *D. isaea* adults. The *D. isaea* colony was started with approximately 1000 wasps obtained from Syngenta Bioline, Oxnard, CA. After a 24 h exposure, plants were removed and held for 14 days until wasps pupated inside the leaf. The leaves were then cut and placed in a separate cage allowing the wasps to emerge. Adult wasps were collected and approximately 50 newly emerged wasps were placed into each wasp oviposition cage daily, maintaining an adult wasp population of approximately 300 individuals per cage. Egg to adult development time was 21 d for *L. huidobrensis* and 24 d for *D. isaea* (at 25 °C and 16:8 (L:D) h photoperiod).

2.2. Host size, body size and sex allocation

We assessed the relationship between host size and population-level sex allocation patterns by taking nine biweekly samples from the *D. isaea* laboratory colony. Each sample consisted of 28–68 randomly selected *L. huidobrensis* larvae that had been attacked by *D. isaea*. The area (length × width) of each attacked larva was measured as an indicator of host size using a dissecting microscope equipped with an ocular micrometer and at 40× magnification. The *L. huidobrensis* larvae and the attached *D. isaea* egg were then transferred to an

artificial mine as described by Heinz and Parrella (1989). Each artificial mine consisted of a small square of moistened card stock with a rectangular hole punched out, resting on a microscope slide. The larva was placed inside the rectangular hole and a coverslip was gently placed over the cardstock and larva. The slide was then placed on a piece of filter paper that was kept moist by periodically added water. The entire setup was placed within a plastic petri dish. The petri dishes were placed in an environmental chamber at 16:8 (L:D) h photoperiod at 25 °C. The relative humidity ranged from 40% to 60%. After 14 days (sufficient to allow wasp eggs to develop to adulthood), the sex of each emerging adult was recorded as well as whether the wasp had died during development. The relationship between host size and host use (host fed, male, female, or wasp died) was examined for each sample with a one-way ANOVA (PROC GLM; SAS Institute, 1989).

To examine the relationship between host size and sex allocation decisions by individual females, we exposed individual mated *D. isaea* females to a heavily infested chrysanthemum plant containing at least 200 second to third instar *L. huidobrensis* for 24 h. This was repeated for 17 females. After the 24 h period, the female wasp was removed and all mines were carefully examined for *L. huidobrensis* larvae that had been attacked (parasitized hosts as well as those which were host fed). Each attacked larva was removed from the mine and its area measured. Each larva with wasp egg was transferred to an artificial mine and reared for 14 days, at which time, the sex of the emerging adult wasp was recorded. Parasitoid eggs that did not yield an adult after this time were recorded as dead. The relationship between host size and host use was examined for each family with a one-way ANOVA (PROC GLM, SAS Institute, 1989).

If a relationship is found between host size and sex ratio it could arise from differential mortality of female wasps on smaller hosts, or it could reflect host size effects on sex allocation decisions by ovipositing females. To distinguish between these two possibilities, we transferred *D. isaea* eggs from small hosts to large hosts and recorded the number of male and female wasps that emerged as well as the number of wasp eggs that failed to develop. As a control, we transferred another set of *D. isaea* eggs from large hosts to large hosts. In both cases, the large hosts to which eggs were transferred were previously parasitized and the eggs that were laid were subsequently removed. If small hosts result in male-biased sex ratios as a result of differential mortality, we would expect to see no difference in sex ratios between the small host to large host transfers versus the large host to large host transfers. These results were tested with a likelihood ratio χ^2 (PROC FREQ; SAS Institute, 1989).

We assessed the relationship between host size and body size by taking a random sample of 366 parasitized

leafminers from our *D. isaea* culture over a 20-week period. Each parasitized larva was carefully removed from its mine with the aid of a dissecting microscope. The size of each parasitized larva was measured as the area (length \times width) with an ocular micrometer at 40 \times magnification. The parasitized leafminer was transferred to an artificial mine as described above. Of the 366 parasitized larvae transferred to artificial mines, 316 (86.34%) successfully yielded an adult wasp. At emergence, wasps were sexed and their head widths were measured. Wasp size was regressed against host size and the homogeneity of slopes of male and female sizes against host size was tested with PROC GLM.

Manipulating individual and group sex allocation patterns. Our ability to improve the sex ratio produced by *D. isaea* depends on how colonies respond to daily shifts in host size. We tested the response of individual females as well as groups of females to daily shifts in host size. Tests with individuals allowed us to examine how individuals alter their sex allocation decisions in response to systematic changes in the host sizes encountered. Tests involving groups of 20–30 females were intended to give an indication of the degree to which sex allocation patterns of mass-reared populations could be altered. We tested the response of individual females by exposing an individual female to hosts in one of the three presentation regimes over a 3-days period: increasing host size, stable host size, or decreasing host size. Mated, 4–5 days-old females were used for this experiment as previous study has shown that *D. isaea* females attain their maximum oviposition rates at this age (Minkenberg, 1989). On day 1, females in all three treatment regimes were exposed to intermediate-sized hosts (1.80–2.20 mm²). Females in the increasing host size treatment were exposed to increasingly larger hosts on days 2 and 3 (2.30–2.80 mm² on day 2; 2.80–3.20 mm² on day 3). Females in the stable host size treatment were exposed to intermediate-sized hosts (1.80–2.20 mm²) across all three days. Females in the decreasing host size treatment were exposed to smaller hosts on days 2 and 3 (1.30–1.60 mm² on day 2; 0.80–1.20 mm² on day 3). All host plants were previously attacked by *L. huidobrensis* for a 3 h period in order to generate a cohort of evenly aged and sized hosts. Each female *D. isaea* was allowed to oviposit for 24 h at which time she was removed. The plant was then isolated and the wasps were allowed to complete development; sex ratio of emerged adult offspring was then recorded. A total of 45 females were exposed to one of the three treatment regimes (15 per treatment). The effect of treatment regime on offspring sex ratio across the three days was tested with a repeated measures ANOVA (PROC GLM; SAS Institute, 1989).

Next, we approximated the effects of host size shifts on mass-reared sex ratios by exposing groups of 30–40 females with an approximately equal number of males

to one of the three treatment regimes as described above. Again, the sex allocation responses of a total of 45 groups of females to one of the three treatments (15 groups per treatment) were tested with repeated measures ANOVA (PROC GLM; SAS Institute, 1989). Each group was exposed to each treatment for a 24 h period.

Imperfect knowledge: weighting current versus past foraging experiences. It is necessary to understand how females weight the relative importance of past versus current encounters when attempting to manipulate ovipositing females so they constantly perceive they are encountering larger than average-sized hosts. To address this issue, we modify the temporal weighting rule model developed by Devenport and Devenport (1994) that weights the influence of a given host encounter based on its recency. The weight assigned to a given host encounter among a series of encounters can be expressed as

$$W_x = (1/t_x^a), \quad (1)$$

where W_x is the weight assigned to a particular host encounter experience out of n such experiences, t_x is the time from that experience to the present, and the exponent a on the value t_x is a constant that weights the relative importance of the recent experience to past experiences. This value can be empirically determined. The inverse of t is the recency of the encounter. A female's temporally weighted estimate of average host size is

$$E_w = \frac{\sum_{i=1}^n W_i x_i}{\sum_{i=1}^n W_i}, \quad (2)$$

where x is the absolute size of a given host. When a (from Eq. (1)) is equal to 0, each encounter experience is unweighted and the estimate of the average host size is simply the arithmetic mean. When a is greater than 0, recent encounters are weighted more heavily than past encounters. When a is less than 0, past experiences are weighted more heavily than recent experiences. For instance, imagine that a foraging *D. isaea* female encounters 4 hosts, one per h, of the following sizes: 0.90, 1.20, 1.80, and 2.20 mm² (going from oldest to most recent host encounter). If a foraging female does not weight experiences according to recency (i.e., $a=0$), she will assess hosts as being large or small on the basis of the arithmetic mean, 1.52 mm². If a *D. isaea* female determines that the next host she encounters is larger than 1.52 mm², she should lay a daughter. Otherwise, she should lay a son. If a female weights recent encounters more heavily than past ones, say $a=1$, she should consider the population average to be 1.77 mm². Such a female should lay sons in hosts smaller than 1.77 mm² and daughters into hosts larger than 1.77 mm². On the other hand, a female who places more weight on past experiences, say $a=-1$, will consider the population average to be 1.30 mm². As the value of a

increases, more weight is placed on more recent encounters.

If we rapidly manipulate the distribution of host size (e.g., rapidly increase the average host size), we expect that ovipositing females will lay a higher proportion of daughters as these new hosts will be perceived as being above average in size. While we do not have a priori knowledge of the temporal weight that *D. isaea* females place on their encounter experiences, we can measure the value of t , the time since a given encounter and, by recording the size of the host and sex of the offspring that was laid in that host, we can judge how the female assessed the size of the host. From this information we can estimate the value of a , indicating the relative weight that females place on recent versus past encounters.

Such information allows us to predict to what degree we can expect to shift sex allocation in this species. We followed individual females as they encountered and attacked up to 20 hosts each day. After each host was attacked, we marked the mine and later measured the hosts and placed them in artificial mines. We then solved Eq. (2) as each host was encountered. This was repeated for a series of values of a at 0.1 intervals from 0 to 2. This generates a predicted threshold value for each subsequent host encounter. If the host size at the next encounter is above the threshold a daughter should be laid; otherwise, a son should be laid. We assume that the value of a that minimizes the discrepancy between predicted and observed sex represents the value of a for that female. The estimated value of a is compared among nine females experiencing one of the three different host exposure regimes: 20 small hosts on day 1, 20 medium hosts on day 2, 20 large hosts on day 3; 10 small hosts followed by 10 medium hosts on day 1, 20 large hosts on day 2; 20 small hosts on day 1, 10 small hosts followed by 10 medium hosts on day 2.

3. Results

3.1. Host size, sex allocation and body size

Within our laboratory culture of *D. isaea*, female progeny emerged from larger hosts than males; the smallest hosts were used for hostfeeding (sex: $F_{3,412} = 163.80$; $P < 0.001$; Table 1). This pattern was consistent across all sample dates despite considerable variation in the average host size available to the colony across the sampling period (group: $F_{8,412} = 5.00$, $P < 0.001$; Table 1) and variation in the magnitude of sex-specific effects of host size across sample dates (sex*group: $F_{24,412} = 1.84$; $P < 0.01$).

The sex allocation response of individual females to host size variation was similar to that of groups of females; of the hosts paralyzed, daughters emerged from

Table 1

Host size and host use decisions by a laboratory colony of *D. isaea* over a 20-week sample period^a

Week	Mean host size (mm ²) ± SE (N)					F ^b	Sex ratio ^c
	Sample	Host fed	Male	Female	Died		
1	1.29 ± 0.07 ^D (68)	0.96 ± 0.05 ^b (27)	1.12 ± 0.07 ^b (21)	2.01 ± 0.15 ^a (14)	1.71 ± 0.11 ^a (6)	29.20	0.60
3	1.60 ± 0.09 ^C (35)	0.93 ± 0.12 ^c (6)	1.46 ± 0.12 ^b (13)	2.06 ± 0.11 ^a (12)	1.71 ± 0.07 ^{a,b} (4)	13.44	0.52
5	1.31 ± 0.09 ^D (28)	0.84 ± 0.09 ^d (5)	1.13 ± 0.07 ^c (15)	2.05 ± 0.05 ^a (5)	1.70 ± 0.06 ^b (3)	32.61	0.75
9	1.63 ± 0.07 ^C (65)	0.99 ± 0.06 ^c (14)	1.54 ± 0.07 ^b (27)	2.21 ± 0.41 ^a (18)	1.75 ± 0.14 ^b (6)	31.77	0.60
11	1.89 ± 0.08 ^B (67)	0.92 ± 0.10 ^c (7)	1.85 ± 0.10 ^b (35)	2.47 ± 0.08 ^a (14)	1.93 ± 0.12 ^b (11)	17.22	0.71
13	1.85 ± 0.07 ^B (60)	0.92 ± 0.09 ^c (7)	1.73 ± 0.07 ^b (28)	2.43 ± 0.09 ^a (18)	1.79 ± 0.10 ^b (7)	32.64	0.61
15	1.64 ± 0.09 ^C (37)	0.95 ± 0.05 ^c (7)	1.50 ± 0.10 ^b (15)	2.17 ± 0.08 ^a (13)	1.58 ± 0.21 ^b (2)	23.94	0.54
17	2.05 ± 0.07 ^A (49)	0.93 ± 0.16 ^c (2)	1.96 ± 0.08 ^b (25)	2.52 ± 0.07 ^a (14)	1.81 ± 0.11 ^b (8)	17.42	0.64
19	1.42 ± 0.09 ^D (39)	0.90 ± 0.09 ^b (7)	1.15 ± 0.08 ^b (18)	2.06 ± 0.12 ^a (11)	1.85 ± 0.14 ^a (3)	24.86	0.62
Overall	1.65 ± 0.03 (448)	0.94 ± 0.03 ^d (82)	1.56 ± 0.04 ^c (197)	2.25 ± 0.04 ^a (119)	1.79 ± 0.04 ^b (50)	163.77	0.62

^a Wasps paralyzing hosts either host fed, laid a male egg, laid a female egg, or the host died before a wasp progeny could complete development. Within each row (sample week), host size values of host fed, male, female and died with different lower case letter superscripts are significantly different (LSD *t* test; $\alpha = 0.05$). With the 'sample week' column, means with different upper case letter superscripts are significantly different (LSD *t* test; $\alpha = 0.05$).

^b All *F*-values were significant at the $\alpha = 0.001$ level.

^c Sex ratio is expressed as proportion male.

larger hosts than males and the smallest hosts were used for host-feeding by adults (sex: $F_{3,800} = 306.10$; $P < 0.001$; Table 2). Again, this pattern was consistent across all 17 families examined despite significant differences in the host size distributions encountered by each female (family: $F_{16,800} = 58.38$; $P < 0.001$; Table 2) and significant differences among families in terms of the magnitude of sex-specific host size effects (sex*family: $F_{48,800} = 2.80$; $P < 0.001$).

The observed relationship between host size and sex ratio is due to female allocation decisions rather than differential mortality of daughters in smaller hosts. *D. isaea* eggs taken from small hosts and transferred to larger hosts yielded significantly more male-biased sex ratios than did eggs transferred from large hosts to large hosts (Table 3; $G = 9.53$; $df = 2$; $P = 0.0085$). Levels of mortality did not differ between the two transfer treatments.

Table 2

Host size effects on the host use decisions made by individual female *D. isaea*^a

Family	Mean host size (mm ²) ± SE (N)					F ^b	Sex ratio ^c
	Family mean	Host fed	Male	Female	Died		
A	1.74 ± 0.07 ^B (21)	1.46 ± 0.11 (4)	1.57 ± 0.03 (3)	1.86 ± 0.09 (12)	1.81 ± 0.10 (2)	2.70	0.20
B	1.46 ± 0.08 ^E (44)	0.72 ± 0.05 ^c (8)	1.20 ± 0.08 ^b (7)	1.73 ± 0.08 ^a (25)	1.65 ± 0.18 ^a (4)	18.00	0.22
C	1.25 ± 0.11 ^F (20)	0.65 ± 0.07 ^c (4)	1.01 ± 0.03 ^b (7)	1.68 ± 0.11 ^a (8)	1.76 ^a (1)	24.14	0.47
D	1.95 ± 0.06 ^A (62)	1.31 ± 0.07 ^c (13)	1.99 ± 0.04 ^b (32)	2.46 ± 0.08 ^a (12)	2.11 ± 0.18 ^b (5)	47.58	0.73
E	0.97 ± 0.03 ^{H,I} (59)	0.68 ± 0.03 ^c (9)	0.97 ± 0.03 ^b (39)	1.39 ± 0.05 ^a (5)	1.09 ± 0.09 ^b (6)	18.78	0.89
F	1.25 ± 0.05 ^F (73)	0.72 ± 0.05 ^c (13)	1.23 ± 0.06 ^b (33)	1.60 ± 0.06 ^a (21)	1.22 ± 0.12 ^b (6)	21.62	0.61
G	1.61 ± 0.03 ^{C,D} (56)	1.35 ± 0.05 ^c (7)	1.58 ± 0.04 ^b (27)	1.75 ± 0.03 ^a (20)	1.73 ± 0.07 ^{a,b} (2)	10.98	0.57
H	1.95 ± 0.04 ^A (72)	1.31 ± 0.04 ^c (3)	1.82 ± 0.04 ^b (41)	2.26 ± 0.05 ^a (25)	1.94 ± 0.44 ^{a,b} (3)	17.88	0.62
I	1.66 ± 0.06 ^{B,C} (42)	1.14 ± 0.05 ^c (7)	1.49 ± 0.05 ^b (14)	2.01 ± 0.05 ^a (17)	1.77 ± 0.20 ^a (4)	30.23	0.45
J	1.10 ± 0.03 ^G (52)	0.82 ± 0.03 ^c (12)	1.09 ± 0.03 ^b (23)	1.36 ± 0.04 ^a (11)	1.23 ± 0.06 ^a (6)	30.99	0.68
K	0.94 ± 0.03 ^{H,I} (59)	0.63 ± 0.04 ^d (15)	0.94 ± 0.03 ^c (26)	1.26 ± 0.03 ^a (13)	1.08 ± 0.05 ^b (5)	58.79	0.67
L	0.92 ± 0.05 ^I (46)	0.58 ± 0.03 ^c (17)	0.99 ± 0.04 ^b (19)	1.42 ± 0.06 ^a (8)	1.09 ± 0.01 ^b (2)	61.86	0.70
M	1.57 ± 0.03 ^D (45)	1.34 ± 0.02 ^c (5)	1.49 ± 0.02 ^b (21)	1.73 ± 0.03 ^a (16)	1.62 ± 0.10 ^{a,b} (3)	20.24	0.57
N	1.58 ± 0.05 ^{C,D} (37)	1.18 ± 0.07 ^c (7)	1.56 ± 0.05 ^b (18)	1.94 ± 0.05 ^a (9)	1.59 ± 0.09 ^b (3)	23.62	0.67
O	1.31 ± 0.06 ^F (63)	0.65 ± 0.05 ^c (13)	1.31 ± 0.07 ^b (28)	1.75 ± 0.06 ^a (17)	1.57 ± 0.20 ^{a,b} (5)	32.26	0.62
P	1.12 ± 0.04 ^G (49)	0.75 ± 0.14 ^c (8)	1.11 ± 0.04 ^b (24)	1.32 ± 0.03 ^a (14)	1.29 ± 0.10 ^{a,b} (3)	11.85	0.63
Q	1.01 ± 0.04 ^H (68)	0.58 ± 0.03 ^c (21)	1.12 ± 0.04 ^b (25)	1.35 ± 0.03 ^a (17)	1.05 ± 0.10 ^b (5)	82.47	0.60
Overall	1.37 ± 0.02 (868)	0.94 ± 0.03 ^d (82)	1.56 ± 0.04 ^c (197)	2.25 ± 0.04 ^a (119)	1.79 ± 0.04 ^b (50)	163.77	0.62

^a Paralyzed hosts were either host fed, received a male egg, received a female egg, or died before the wasp progeny could complete development. Within each row (sample week), host size values of host fed, male, female and died with different lower case letter superscripts are significantly different (LSD *t* test; $\alpha = 0.05$). Within the 'Family mean' column, means with different upper case letter superscripts are significantly different (LSD *t* test; $\alpha = 0.05$).

^b All *F*-values, except for family A, were significant at the $\alpha = 0.001$ level.

^c Sex ratio is expressed as proportion male.

Table 3
Distinguishing sex allocation decisions from sex-specific differential mortality

Transfer direction	Mean host size (mm ²) ± SE		Outcome		
	Original	New	Male	Female ^a	Died ^b
Small to large	1.25 ± 0.03	2.95 ± 0.01	48	28	10
Large to large	2.83 ± 0.03	2.94 ± 0.01	34	51	7

^a The proportion of male and female offspring in the two treatments was significantly different (G test = 8.68, 1 df, $P < 0.005$).

^b The proportion of eggs that failed to develop was not significantly different between the two transfer treatments (G test = 0.87, $P > 0.10$).

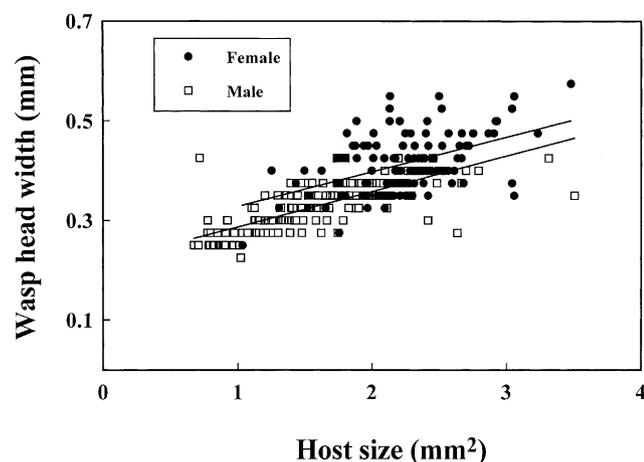


Fig. 1. Relationship between *D. isaea* male (open squares) and female (closed circles) body size and host size of *L. huidobrensis* larvae. The regression equations between wasp head width and host area did not significantly differ between males and females (see text).

The size of the host when it was paralyzed was positively correlated with the size of both male and female *D. isaea* offspring (Fig. 1; (wasp head width) = 0.1961 + 0.0901*(host size), $r^2 = 0.5962$; $t = 21.53$; df = 1314; $P < 0.0001$). While females tended to develop in larger hosts, the relationship between host size and parasitoid size did not significantly differ between males and females (test of different intercepts: $t = 1.93$; df = 1; $P = 0.0541$; test of different slopes: $t = 0.19$; df = 1; $P = 0.8532$).

3.2. Manipulating individual and group sex allocation patterns

Individual females exposed to either increasing, decreasing, or stable host size distributions over three days produced substantially different offspring sex ratios (Fig. 2; between individual exposure effects $F_{2,42} = 90.67$; $P < 0.001$). Exposure day did not have a significant effect on the sex allocation response of individual females (day effect: $F_{2,84} = 0.70$; Greenhouse–Geisser adjusted $P = 0.4981$). This indicates that the response of females to increasing host sizes was symmetrical to the responses of females to decreasing host sizes over the three days of the experiment. However, the interaction between exposure day and treatment was highly significant (Fig. 2;

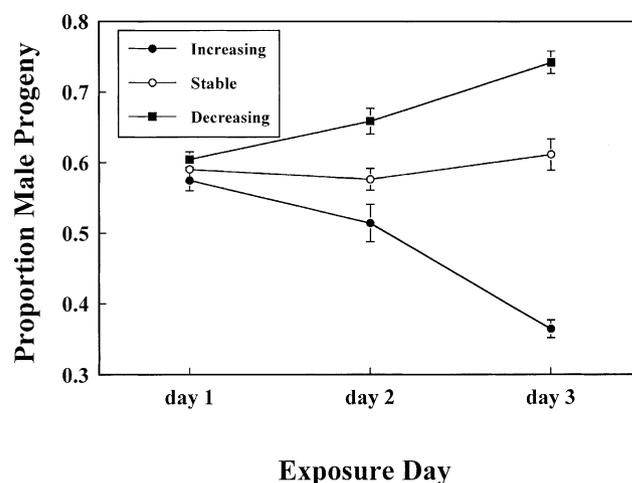


Fig. 2. Sex allocation patterns (mean ± 1 SEM) of individual females exposed to systematically increasing (closed circles), decreasing (closed squares), or stable (open circles) host size distributions. $N = 15$ females for each exposure treatment.

day*treatment: $F_{4,84} = 25.82$; Greenhouse–Geisser adjusted $P < 0.001$). On the first exposure day, females across all treatments produced sex ratios of approximately 60% male. By day 3, females exposed to decreasing host sizes produced 74% male offspring whereas females exposed to increasing host sizes produced 36% male offspring. Females experiencing a stable distribution of host sizes across the three days consistently produced sex ratios of around 60% male.

Similar to individual females, groups of females exposed to either increasing, decreasing, or stable host size distributions produced significantly different sex ratios (Fig. 3; between group exposure effects: $F_{2,42} = 28.30$; $P < 0.001$). Within groups of females, exposure day did not have a significant effect (day effect: $F_{2,84} = 3.05$; Greenhouse–Geisser adjusted $P = 0.0537$) indicating that the response of groups to increasing host sizes was symmetrical to the responses of groups to decreasing host sizes over the three days of the experiment. The interaction between exposure day and treatment was highly significant (Fig. 3; day*treatment: $F_{4,84} = 11.74$; Greenhouse–Geisser adjusted $P < 0.001$). On the first exposure day, groups of females across all treatments produced sex ratios of approximately 63% male. By day 3, groups of females exposed to decreasing host sizes

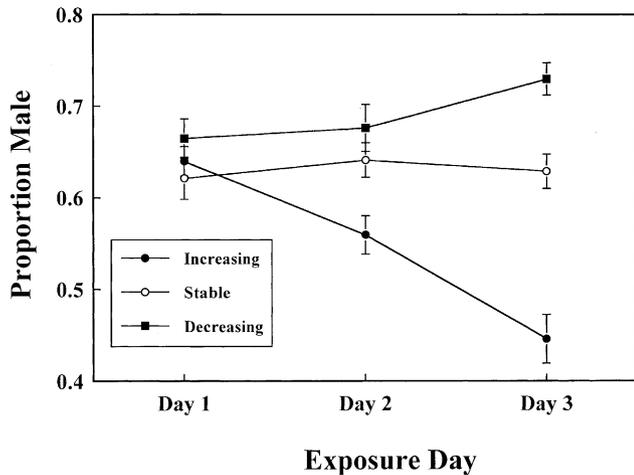


Fig. 3. Sex allocation patterns (mean \pm 1 SEM) of groups of 30 females exposed to systematically increasing (closed circles), decreasing (closed squares), or stable (open circles) host size distributions. $N = 15$ groups for each exposure treatment.

produced 73% male offspring whereas groups exposed to increasing host sizes produced 45% male offspring. Groups experiencing a stable distribution of host sizes across the three days consistently produced sex ratios of approximately 63% male.

3.3. Imperfect knowledge: weighting current versus past foraging experiences

Our empirical estimates of the memory weighting parameter a ranged from 1.0 to 1.4 and did not differ appreciably among the three exposure treatments (20 small–20 medium–20 large: $a = 1.2, 1.2, 1.0$; 20 small–10 medium–10 large: $a = 1.4, 1.3, 1.1$; 10 small–10 medium–20 large: $a = 1.3, 1.3, 1.0$) indicating that ovipositing females assess mean host size on the basis of more recent host encounters rather than past host encounters. The relative weight placed on recent versus old encounters appears to be constant, at least over the range of exposure regimes tested. The predicted sex ratios (which are determined by the weighting parameter, the host sizes encountered and the elapsed time between encounters) did not significantly differ from the observed sex ratios; neither the predicted nor the observed sex ratios differed among the exposure treatments either overall (Fig. 4) or within each exposure period (Fig. 5).

4. Discussion

Our study clearly shows that we are able to modify not only the sex ratios produced by individual females but also by small groups of females by manipulating the size distribution of exposed hosts. We are able to do this

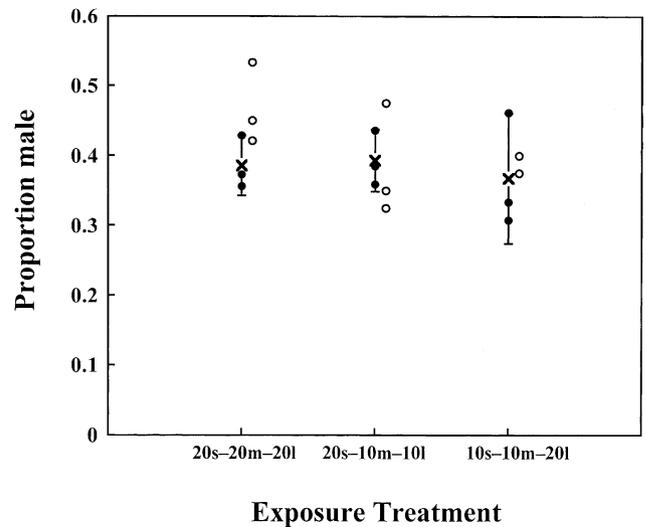


Fig. 4. Overall predicted (closed circles) and observed (open circles) sex ratios for females exposed to hosts in one of the three exposure regimes: 20 s–20 m–20 l = females exposed to 20 small hosts on day 1, 20 intermediate-sized hosts on day 2 and 20 large hosts on day 3; 20 s–10 m–10 l = females exposed to 20 small hosts on day 1, 10 intermediate-sized hosts followed by 10 large hosts on day 2; 10 s–10 m–20 l = females exposed to 10 small hosts followed by 10 intermediate-sized hosts on day 1, and 20 large hosts on day 2. Three females were followed for each of the three exposure regimes (two values of observed sex ratios in the last exposure treatment overlapped). The 'x' represents the mean predicted value \pm 95% confidence limit.

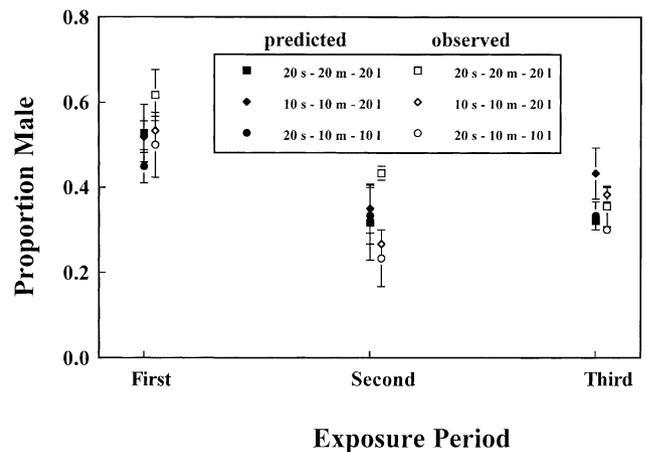


Fig. 5. Overall predicted sex ratios (closed symbols) and observed (open symbols) sex ratios produced by females exposed to hosts in one of the three exposure regimes: 20 s–20 m–20 l = females exposed to 20 small hosts on day 1, 20 intermediate-sized hosts on day 2 and 20 large hosts on day 3; 20 s–10 m–10 l = females exposed to 20 small hosts on day 1, 10 intermediate-sized hosts followed by 10 large hosts on day 2; 10 s–10 m–20 l = females exposed to 10 small hosts followed by 10 intermediate-sized hosts on day 1 and 20 large hosts on day 2. Means are presented for each oviposition period. Three females were followed for each of the three exposure regimes.

because females differentially allocate daughters to larger hosts and sons to smaller hosts and the assessment of small versus large is relative to prior host encounters

rather than absolute. While the relationship between host size and wasp size of *D. isaea* did not differ between males and females, male and female fitness may be differentially correlated with body size. A similar relationship between host size, wasp size, and wasp fitness correlates (e.g., male mating ability and fecundity) has been documented in *D. begini* (Heinz, 1991; Heinz and Parrella, 1989). We were also able to modify the sex allocation patterns of females because, although females appear to weight recent host encounters more heavily than past encounters, past encounters do have an influence on a female's assessment of average host size. We did not estimate a in situations where females encounter decreasing host size averages over time, primarily because we were interested in how wasps adjust sex allocation in response to increasing host size for mass-rearing purposes. However, the sex allocation responses of individual females, as well as groups of females, encountering progressively smaller hosts suggest that more weight is placed on recent encounters.

We were able to change the sex ratio produced by groups of females from 64% male to 45% male and we were able to drop the sex ratios of individual females to 36% male. If this degree of sex ratio shift can be realized at a commercial insectary level, biocontrol practitioners would only have to release 65% of the total number of individuals and still attain the same level of *Liriomyza* spp. control by *D. isaea*. *D. isaea* adult females live considerably longer than the three days treatment regime suggested here (10 d on average at 25 °C; Minckenberg, 1989). While we did not examine treatment regimes other than three days, it may be possible to extend the duration of the treatment to encompass a greater fraction of the adult female's lifespan either by more finely subdividing the host size categories between small and large or by increasing the amount of time before the next set of larger hosts is presented. Alternatively, wasps could be given access to only large leafminer larvae after they have been exposed to increasing host sizes for three days. This would minimize the overall decrease in the proportion of daughters produced after the modification period. Rearing modifications at the insectary scale to induce such sex ratio shifts may be relatively inexpensive, particularly if they primarily involve more stringent controls to produce evenly aged host distributions and a standardized schedule to expose each wasp culture to increasingly large sets of hosts. Such rearing modifications are not expected to involve more plant or host material, more rearing space, or increased labor costs. If protocol changes for the insectary are relatively inexpensive, most of the cost savings resulting from the change in sex ratio can be directly translated into cost reduction for the biocontrol practitioner. Ideally, this will encourage the more widespread use of *D. isaea* as a viable pest control strategy.

Our estimates of memory and relative host size assessment suggest that mass-rearing strategies might be further modified to produce even more female-biased sex ratios. If the host size distribution presented to the wasps is quickly shifted from small to medium, our predictions suggest that sex ratios of approximately 30% male could be expected. This would cut costs to 46% of those incurred under conventional mass-rearing programs. More rigorous host-presentation schemes may result in even greater reductions in sex ratio and consequently greater effective reductions in cost.

Using a similar host size manipulation procedure, Heinz (1998) was also able to generate substantially more female-biased sex ratios in mass-reared cultures of the boll-weevil parasitoid, *Catolaccus grandis* (Burks) (Hymenoptera: Pteromalidae). This suggests that the ability to artificially induce more female-biased sex allocation in mass-reared cultures of parasitoids has widespread potential. This technique holds the greatest promise of success in solitary parasitoids of hosts that cease to develop after parasitism (idiobionts) (Godfray, 1994; King, 1989; Waage, 1982; Wellings et al., 1986). Fitness of gregarious parasitoids (those where more than one offspring routinely complete development in a single host) is determined largely by the number of offspring per host rather than a direct effect of host size. Females attacking hosts that continue to grow after parasitism (koinobionts) are less likely to exhibit host-size dependent sex allocation behavior because they are less likely to be able to predict the final amount of host resources available for their offspring. In a literature survey, King (1989) showed that idiobionts tended to exhibit host-size dependent sex allocation more frequently than koinobionts; however, some solitary koinobionts may be able to assess host quality differences particularly if they are correlated with different host species (e.g., Chau and Mackauer, 2000). Yet, not all species exhibiting host-size advantage effects appear to be capable of within individual shifts in sex allocation. Jones (1982) showed that female *Heterospilis prosopidis* Viererck (Hymenoptera: Braconidae) were unable to adjust their offspring sex ratios in response to changes in within-generation host-size distribution shifts. van Dijken et al. (1991) found that female *Epidinocarsis lopezi* (DeSantis) (Hymenoptera: Encyrtidae), a parasitoid of the cassava mealybug, lay mostly males in small hosts regardless of their relative abundance. Population sex ratios in this species are largely determined by the relative proportion of small and large hosts. Similarly, field sex ratios of *Metaphycus stanleyi* Compere (Hymenoptera: Encyrtidae) are determined largely by the available size distribution of hosts (Bernal et al., 1998). Many currently available natural enemies from insectaries are solitary (e.g., Hunter, 1997) and are potential candidates for improved sex ratios through the

modified mass-reared protocols presented here. However, given some of the exceptions described above, the host-size dependent sex allocation behavior of each mass-reared species needs to be tested.

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