

Male-biased Sex Ratios in Laboratory Rearings of Gypsy Moth Parasitoids

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Abstract

Male-biased sex ratios in laboratory colonies of parasitic wasps used in biological control are harmful because they can prevent the establishment of introduced species or hinder commercial production of species used for augmentative control. Over 20 species of parasitic wasps, most in the families Ichneumonidae and Braconidae, were imported and released against the gypsy moth, *Lymantria dispar* (L.), during 1971-1999. We checked quarantine records to see how often this problem developed in laboratory rearings of gypsy moth parasitoids. In the Ichneumonidae, incoming sex ratios from field collected material were usually 50:50 or female-biased, and marked shifts towards male-biased sex ratios were not observed in outgoing shipments following laboratory rearings (Fig. 1). In the Braconidae, incoming shipments were also about 50:50 or female-biased, but four species, *Aleiodes indiscretus*, *Apanteles* sp., *Cotesia melanoscelus*, and *Glyptapanteles flavicoxis*, showed marked shifts towards male-biased sex ratios (Fig. 2).

The rest of my presentation will focus on sex ratio studies in *G. flavicoxis*, a gregarious larval parasitoid. This species, collected from Indian gypsy moth, *Lymantria obfuscata* (Walker), was selected for study because (1) it can be reared in large numbers using relatively few hosts, (2) it has high dispersal power and

host finding ability at low gypsy moth densities, and (3) it might have potential for inundative releases directed at specific gypsy moth populations (Krause et al. 1991). In fact, *G. flavicoxis* was integrated into a successful urban forest IPM program for gypsy moth in Virginia, but Bt and releases of *C. melanoscelus* were used also, so it is hard to say how much it lent to the suppression effort (Ticehurst and Finley 1988). We report here the results of monitoring sex ratios in a new colony from India, an experiment testing the effects of holding and mating temperatures for adults on sex ratios in their progeny, a second experiment testing the effects of offering hosts to mated females with or without a post-mating rest period, and a third experiment testing the effects of the number of times a female has copulated.

Methods and Materials

Monitoring New Colony. Pedigrees were created for all but the F₁ generation, because many P₁ founders had emerged *en route* and could have mated earlier. In this and ensuing studies, cocoons of *G. flavicoxis* were isolated in gelcaps, and emergees were sexed and held in separate cages with honey and water, so that virgin females were available for controlled matings. In this study, adults were stored at 16°C until mating. Each pair of adults was placed in a 7-cm shell vial containing a droplet of honey and a cotton plug. After mating, females were given a 15-24 h rest period, then placed in

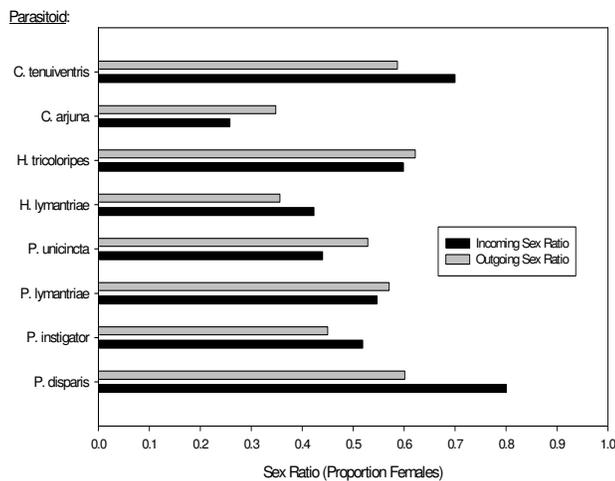


Figure 1.—Incoming and outgoing sex ratios in ichneumonid parasitoids of the gypsy moth.

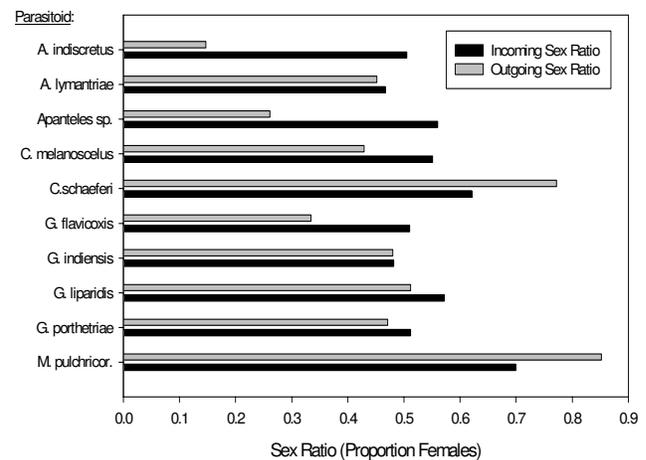


Figure 2.—Incoming and outgoing sex ratios in braconid parasitoids of the gypsy moth.

a sting unit with five unparasitized larvae (instars IV-V) of *L. dispar* for 48 h. In this and ensuing studies, parasitized hosts were fed high-wheat germ artificial diet and held at 25° C; 14:10 (L:D) photoperiod, and 50-60% RH. We monitored the colony for five generations, observing the overall frequencies of sex ratios, and compared sex ratios between generations and between sib-mated and outcrossed females. We also tested for correlation between sex ratios among a pair's progeny and those of the parental generation.

Effects of Storage and Mating Temperatures. After emergence from their cocoons, virgin adults of *G. flavicoxis* were stored at 13° C or 16° C for 16-24 h. Virgin males and females of *G. flavicoxis* that had been held for 16-24 h at 13° C or 16° C were placed in holding cages at 20° C or 25° C under fluorescent lights for three hours. Fifteen males and five females were placed in the mating cage associated with each combination of holding × mating temperature; then, each female was placed in a sting unit containing 10 fourth-instars of *L. dispar* for 48 h. The sex ratio (expressed as % females) of each female's progeny was determined. Data were subjected to two-way ANOVA with holding and mating temperatures as grouping variables.

Effects of Post-Mating Rest Period. Emerging male and female parasitoids were stored separately in holding cages (described above) containing droplets of honey and atomized with distilled water at 16° C until ready for mating trials. Each pair of adults were placed in a 7-cm shell vial containing a droplet of honey and a cotton plug. Vials were watched carefully, and the pair separated as soon as the first copulation was completed. After the observation period, usually 1-60 min, one half of the females were placed individually in sting units containing 10 unparasitized 4th-instars of *L. dispar* for 24 h, and transferred to fresh sting units daily until the female died. The remaining females mated on that date were placed in similar sting units on the following day (23-25 h later) and treated the same thereafter. The experiment was run twice with colony founders received from India in 1999 and 2000. Sex ratios compiled over a female's lifetime were subjected to two-way analysis of variance with treatment and year as grouping variables.

Effects of Multiple Matings. Matings were conducted by placing three males and one virgin female in a 7-cm shell vial containing a droplet of honey and a cotton plug. Extra males were removed once a copulation was observed to negate any genetic variation that might be caused by some females mating with more than one male. Mated pairs were moved to different trays as

successive matings were observed. After the observation period, usually 90-120 min, males were removed, and vials containing mated females marked with the number of times the female therein had copulated. No females were allowed to copulate over four times. Each female had a 15-17 h rest period prior to host exposure, then was placed in a sting unit with 5 unparasitized 5th-instars of *L. dispar* for 48 h. Sex ratios of progeny for each female were recorded.

Results and Discussion

Monitoring of New Colony. Figure 3 shows the frequency distribution of sex ratios over five generations. Sex ratios (stated as % females) ranged from 0 to 68%, averaging 9.3% (SEM = 1.1%), and were not normally distributed but highly skewed at the lower end. Nearly 60% of the mated pairs had no female progeny, and only 28% had sex ratios \pm 5%. The usual host of *G. flavicoxis* is *L. obfuscata*, so *L. dispar*, though a close relative, is a novel host. The host switch might have induced *G. flavicoxis* females to allocate fewer female eggs to *L. dispar*. Sex ratios differed among generations, averaging 13-17% in the F₁, F₂, and F₄ generations, but <4% the F₃ and F₅. Proportions of parental females yielding progeny of both sexes followed a similar trend. The reason for these differences is unknown. When grouped by cross type (sib-mating or outcross), sex ratios did not differ significantly. We looked at the relationship between sex ratios in filial (3-5) and parental (2-4) generations by regressing the sex ratio of each pair's progeny against those of its parents. A model with two independent variables (sex ratios of the maternal and paternal families) and one dependent variable (sex ratio of pair's progeny) was not statistically significant.

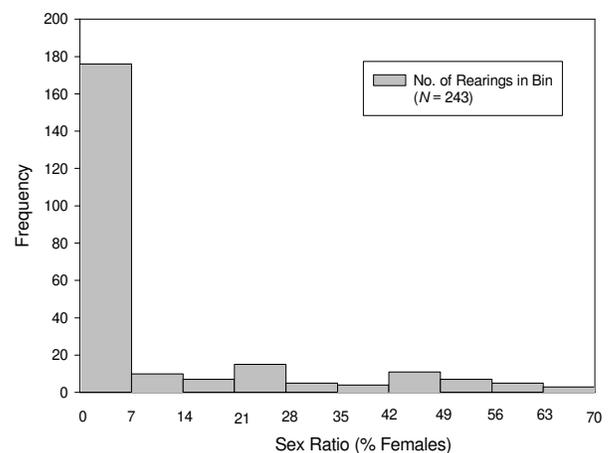


Figure 3.—Histogram for overall distribution of sex ratios in *G. flavicoxis* across five generations.

Effects of Storage and Mating Temperatures.

Temperatures in holding or mating cages for parental adults did not affect sex ratios in their progeny (Fig. 4). Sex ratios were highly variable, because about half of the females had no female progeny, presumably because they failed to mate. In most species of parasitic wasps, unfertilized (haploid) eggs result in male progeny, while fertilized (diploid) eggs result in female progeny, causing unmated females to produce only male rather than mixed progeny.

Effects of Rest Period and Year. Sex ratios for total progeny produced over a female’s lifetime differed between treatments ($F = 4.35$; $df = 1,31$; $P < 0.05$), being over twice as high in females having a rest period than in those which did not (Fig.5). Year and treatment-year interaction effects on sex ratio were not significant. Six of the 16 females without a rest period failed to produce any female progeny, whereas only one of 19 given a rest period failed to do so. These proportions differed significantly (Fisher’s exact test, $P = 0.024$). Because all of the females in this experiment, had copulated before the tests began, these results indicate that copulation does not always ensure that a female is impregnated and that *G. flavicoxis* might need a period of inactivity following copulation.

Effects of Number of Copulations. ANOVA showed that the number of times (1-4) a female had copulated did not appear to influence the sex ratios in her progeny. Figure 6 suggests that sex ratios might tail off when the number of matings exceeds two, so we grouped them into only two categories (1-2 and 3-4) and attempted to retest with a one-way ANOVA. With only two groupings, the Kolmogorov-Smirnoff normality test failed ($P = 0.002$), so we subjected the data to Kruskal-Wallis one-way ANOVA on ranks. The difference in rankings was statistically significant ($H = 4.302$; $df = 2$; $P = 0.038$). The median sex ratio for those females mating 1-2 times ($n = 20$) was 36.3%, whereas that for females mating 3-4 times ($n = 19$) was only 1.3%. These results suggest the sex ratio of *G. flavicoxis* might be adversely affected when the number of copulations exceeds two.

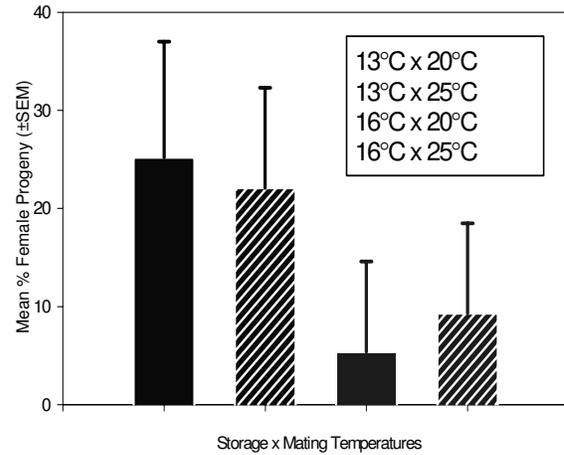


Figure 4.—Effect of storage and mating temperature on sex ratio of *G. flavicoxis*.

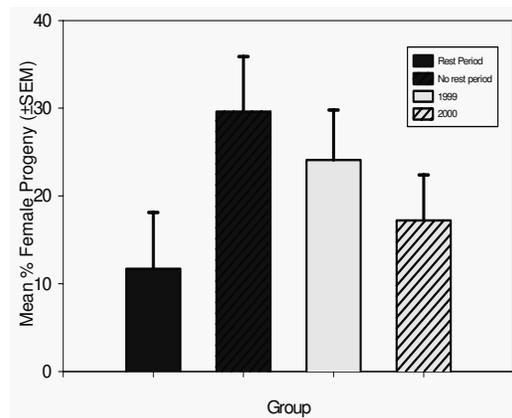


Figure 5.—Effect of rest period and year on sex ratio of *G. flavicoxis*.

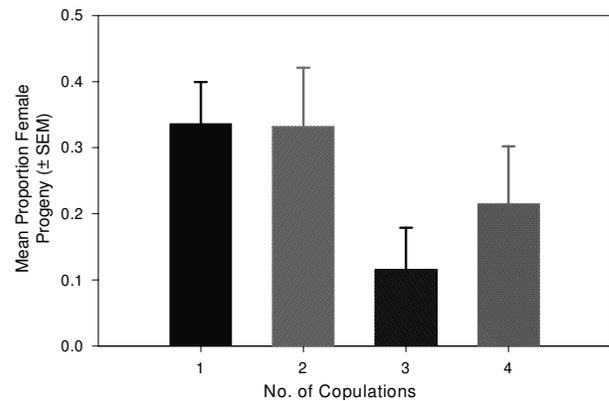


Figure 6.—Effect of number of copulations on sex ratio in *G. flavicoxis*.

Summary and Conclusions

Sex ratios were very low in the new colony, suggesting the host switch (*L. obfuscata* to *L. dispar*) was responsible, but other factors cannot be ruled out. Sex ratios differed among generations, but not in a consistent pattern. Holding and mating temperatures for parasitoid adults did not affect the sex ratio of their progeny. Females given a post-mating rest period had higher sex ratios in progeny than those which did not, but over two copulations appeared to depress sex ratios in their progeny. While the best treatments enhanced the sex ratio, the sex ratios were lower than those in incoming shipments. More work is needed.

Acknowledgment

We thank Dr. Gujjanadu Ramaseshiah for providing field collected *Glyptapanteles flavicoxis*.

References

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