

INSEMINATION CAPABILITY OF MALE *PTEROMALUS*
VENUSTUS (HYMENOPTERA: PTEROMALIDAE),
A GREGARIOUS PARASITOID OF *MEGACHILE ROTUNDATA*
(HYMENOPTERA: MEGACHILIDAE)

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ABSTRACT

Inseminative ability in *Pteromalus venustus*, an arrhenotokous parasitoid presumed to have evolved under Local Mate Competition, was studied by recording the progeny production of a series of six virgin females presented to males at hourly intervals. Most males did not reach the upper limits of their inseminative abilities: Three-quarters of the males inseminated at least four females, twice the ratio of females-to-males in an average brood (2:1). There was no evidence of sperm depletion: The few uninseminated females did not tend to be those presented later in the mating sequence, and a comparison of progeny production by female position in the mating sequence showed no tendency for females presented later to produce fewer females than those presented earlier. The data suggest that males can inseminate at least twice the number of females that normally share a host with them. The breeding system of this species may be moving towards panmixis because of recent selection pressures caused by domestication of its primary host, the alfalfa leafcutting bee.

INTRODUCTION

Among the Hymenoptera Parasitica, many species of gregarious parasitoids exhibit primary sex ratios that are strongly biased towards females. To explain such unexpected deviations from Fisher's (1958) predictions of equal investment in the sexes, Hamilton (1967) proposed the theory of Local Mate Competition (LMC). Under LMC inbreeding is common because brothers

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frequently compete to inseminate their sisters before the latter disperse from their natal host. Under such circumstances, each host should receive only as many males as are necessary to inseminate their sisters that share the same host. If males are capable of multiple inseminations over the usual period of female emergence, the sex ratio of siblings that share a host should be biased towards females.

Male multipotency is commonly assumed (e.g., Green et al. 1982), but less commonly tested. Three recent reviews of empirical studies (van den Assem 1986, King 1987, Ridley 1988) agree that male parasitoids are usually multipotent, but note that their inseminative abilities are influenced by such factors as male size, age, and rate at which females are offered. Here I examine the ability of male *Pteromalus venustus* Walker (Pteromalidae) to inseminate their sisters under conditions approximating those found in natural populations.

Pteromalus venustus has several attributes which are indicative of a species under LMC. It is an external, gregarious parasitoid of the prepupal to late pupal stages of leafcutting bees (Megachilidae) (Tepedino 1988b). Bees are attacked in their nests as the female parasitoid pierces the leaf sheaf and cocoon with her ovipositor, anesthetizes the host and oviposits upon it. The number of eggs deposited per host varies: Hobbs and Krunic (1971) reported an average of 32.3 eggs, Whitfield and Richards (1985) found 17.4 adults per host, and I observed between 8.3 and 14.0 eggs per host (Tepedino 1988b). The variation in these estimates may be due to differences in host and/or parasitoid size.

Mating occurs before females disperse. Hobbs (1968) reported that males emerged, on average, one day before females but Tepedino (1988a) found no evidence for protandry. Females may mate: 1) prior to emerging from the host cell (Tepedino 1988a); 2) immediately upon emerging from the host cocoon (Hobbs and Krunic 1971); 3) within hours of emergence on exposed faces of leafcutting bee nest blocks (personal observation). Mating is rapid (a matter of seconds), and females rarely mate more than once (Hobbs and Krunic 1971).

The sex ratio of emergent adults has been reported as 1:1 (Hobbs and Krunic 1971, Whitfield and Richards 1985). In the latter case the sex ratio actually varied among sites and years from 1:1 to 3.5 females per male, with a mean of 1.5 females per male.

In my studies (unpublished), the sex ratio also varied from unity to >3 females per male, but was most commonly in the range of 1.5–2 females per male. Thus *P. venustus* has many of the characteristics of a species under LMC.

METHODS

Pteromalus venustus individuals were obtained from stock cultures maintained at the Bee Biology and Systematics Laboratory, Logan, Utah. All rearings and experiments were conducted at 29°C in the dark. Parasites were separated into unisexual groups at the pupal stage, reared to eclosion and then held individually in glass petri dishes for 24 hours. Individuals were fed 50% honey solution which was applied to a cigarette filter (honey plug) fitted into an arm in the side of the dish.

After 24 hours, six virgin females were introduced, one at a time at hourly intervals, into each dish containing a male. One-hour intervals were chosen because they roughly simulate the rate at which each male would encounter his emerging, virgin sisters in the presence of his competing brothers. At the end of the hour, the female was placed in an individual glass vial whose top was modified to hold a honey plug. Each vial contained a live, immature alfalfa leafcutting bee in its cell. Cells had been radiographed (Stephen and Undurraga 1976) prior to use so that those containing dead immatures could be removed. Bee cells were taken from storage at 4°C and held at room temperature (~25°C) for 24 hours before being used. A female parasitoid was held with a bee cell for 24 hours; the cell was then removed and replaced with a fresh one, and the honey plug was replenished. Each replication was terminated when either the female expired or ten bee cells had been presented to her.

After removal, bee cells were held in individual gelatin capsules until either parasites or an adult bee emerged. Because *P. venustus* is haplodiploid, male, but not female, progeny can be produced by uninseminated females. Thus, female parasites were considered to have been inseminated if they produced one or more female progeny during their lifetime. Cells from which no insect had emerged after eight weeks were dissected and their contents recorded.

Data were analyzed using paired t-tests, analysis of variance (ANOVA), and linear regression. All percentages were converted

by arcsin transformation for analysis but are reported as untransformed values.

RESULTS

Before examining how likely *P. venustus* males are to completely inseminate their sisters, the average sex ratio per host must be established. Broods from all hosts parasitized in the subsequent studies were used to estimate the sex ratio. On average, hosts contained between two and three females per male depending upon whether broods with 100% male progeny were included (Fig. 1). Approximately 50 percent of all broods contained four or more females per male and about 70 percent had 60 percent or more females.

Three aspects of male virility were investigated: the ability of a male to provide some sperm to each of six females presented successively at hourly intervals; the presence of a male refractive period as suggested by the pattern of occurrence of uninseminated females in the presentation sequence; and sperm depletion in indi-

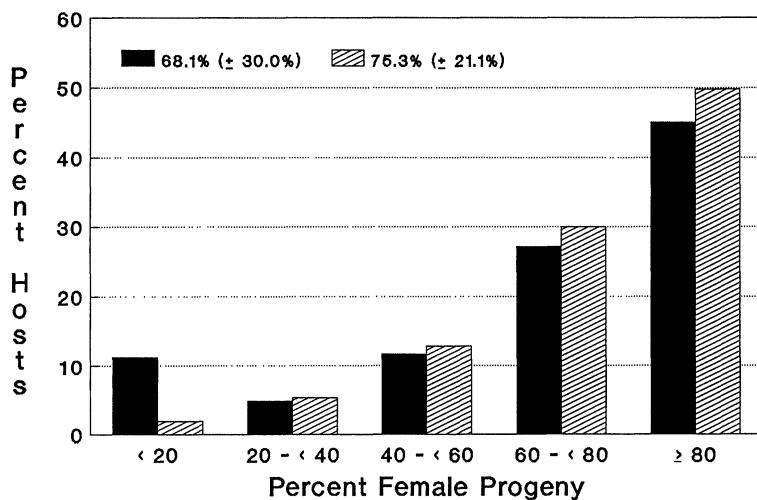


Figure 1. Frequency distribution of hosts by percent female *P. venustus* they contained. Black bars represent all hosts ($N = 291$), hatched bars represent all hosts that contained at least one female ($N = 263$). Means (\pm sd) presented for each group.

vidual females as indicated by the pattern of occurrence of hosts with only male progeny in the parasitization sequence.

Approximately half of all males inseminated all six females with whom they were paired, and 75% serviced at least four females (Table I). Only one male (#1) inseminated as few as two females and his performance may have been unjustifiably impugned by two of his mates who did not parasitize any hosts, and who lived only three and five days (as opposed to average female lifespan of 7.1 ± 2.1 SD days, $N = 120$). Thus, almost all males seem capable of supplying some sperm to at least four females within a six hour period and there is no indication of a prolonged (>1 hour) refractory period.

If males had reached the upper limits of their inseminative ability at four or five females, then there should have been a disproportionate number of uninseminated females among the last three females presented. However, this was not the case: Of eleven females that produced only male progeny, eight were among the first three females to be presented to their respective mates (3 #1s, 3 #2s, 2 #3s), but only three were among the last three to be presented (2 #4s, 1 #5). Thus, uninseminated females did not tend to be those paired with males later in the presentation sequence.

Females need not be uninseminated to demonstrate that males have become depleted of sperm; underinsemination (transfer of fewer than average sperm per mating) of some or all of a male's mates may also attest to sperm depletion. Underinsemination was tested for in two ways. First, I reasoned that if some of a male's mates were underinseminated it would likely be those presented later in the mating sequence. Therefore, if sperm was depleted, later-mated females should have produced fewer female progeny than those produced by earlier-mated females. To test this hypothesis under standardized conditions, only data for the nine males that inseminated all six of their mates (Table I) were used. The data were analyzed in two ways using a randomized block ANOVA: by total number, and percent, of female progeny produced by each of the six females (Table II). In neither analysis was there an indication of sperm depletion: the number ($F_{5, 40} = 2.22$) and percent ($F_{5, 40} = 1.02$) of female progeny produced by later females in the sequence was indistinguishable from that produced by earlier females ($P > 0.05$, both tests).

Table I. For each male *Pteromalus venustus*, the number of mates that parasitized at least one host, the number of mates producing some female progeny, and the total number of male and female progeny produced.

Male #	Number of Mates		Number		
	producing	progeny	♂	♀	Dead larvae
1	4	2	102 ^a	17	0
2	6	5	28 ^a	93	1
3	5	5	52	92	19
4	6	6	42	125	1
5	6	6	61	68	5
6	6	6	128	77	0
7	5	3	213 ^a	71	2
8	6	3	186 ^a	128	7
9	5	4	71 ^a	74	23
10	6	6	27	131	8
11	6	6	50	249	15
12	6	6	61	189	11
13	6	6	117	134	21
14	4	4	31	63	26
15	5	4	80 ^a	93	21
16	4	4	20	47	17
17	4	4	48	74	26
18	6	6	44	145	2
19	6	5	37 ^a	120	15
20	6	6	37	117	56
Mean	5.4	4.9	71.8	105.4	13.8
(± SD)	(0.8)	(1.3)	(52.9)	(51.7)	(13.5)

^a Includes male progeny produced by uninseminated females

Table II. Mean number and percent (\pm SD) of female progeny produced by females according to their position in the mating-presentation sequence. Only data for males that inseminated all six of their mates (Numbers 4, 5, 6, 10, 11, 12, 13, 18, 20) are included.

Mean (\pm SD)	Order of Female Presentation					
	1	2	3	4	5	6
Number females	21.4 (15.4)	21.6 (10.2)	27.8 (19.8)	19.0 (16.1)	24.3 (12.8)	23.1 (11.3)
Percent females	75.2 (26.0)	69.9 (12.1)	69.3 (18.6)	59.0 (25.8)	76.0 (13.9)	66.2 (30.4)

A second way underinsemination might occur is if each female received less sperm than needed to fertilize multiple broods. For example, males might be incapable of completely replenishing their sperm in one hour. If limited amounts of sperm were being passed to each female, irrespective of her place in the presentation sequence, then the number of female progeny from the last host parasitized by each female should be lower than that from the first host parasitized. To test this, only inseminated females that parasitized three or four hosts were used. (Too few females parasitized five or six hosts for analysis (Fig. 2), and females that parasitized two hosts, though numerous, each parasitized too infrequently for meaningful comparison.) As a precaution, two preliminary tests were applied to be sure there was no association between number of hosts parasitized and position of female in the mating sequence. First, an ANOVA of number of hosts parasitized by female position was conducted; results were non-significant ($F_{5, 114} = 1.03$, $P > 0.40$). And, second, the average position number of three-host and four-host females was compared with that expected if all mating positions were equally represented. The average mating position for three-host females was 3.4, and that of four-host females was 3.7, hardly distinguishable from the expected value of 3.5.

The number of female, male and total progeny produced in the first and last cells parasitized was compared using paired student's t-test (Table III). In the three-host group no significant differences were found in the total number of progeny ($t = 0.17$), the number of female progeny ($t = 0.58$), or in the number of male progeny

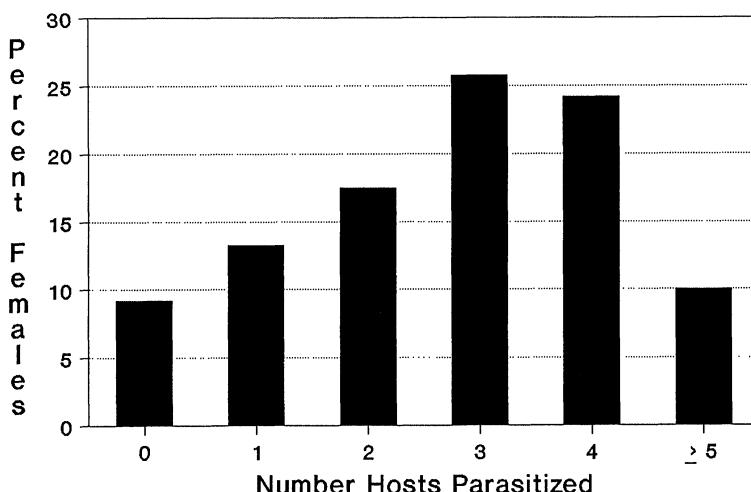


Figure 2. Frequency distribution of percent female *P. venustus* by the number of hosts they parasitized.

($t = 1.07$, all d.f. = 27, $P > 0.25$). However, in the four-host group, there were significantly fewer total progeny ($t = 2.49$), and significantly fewer females ($t = 2.64$, both d.f. = 22, $P < 0.02$) in the last host parasitized than in the first host parasitized. There was no significant difference for males ($t = 0.65$, d.f. = 22, $P > 0.50$). Thus, in contrast to females that parasitized three hosts, there is some indication that those that parasitized four hosts experienced either sperm or egg depletion.

The data also give an idea of potential variability in male reproductive success. Large differences in the number of female progeny sired were evident. Table IV shows the mean number of female and male progeny produced per female mate for each of the nine males that inseminated all six females presented to them. These differences in number of female progeny were highly significant (ANOVA, $F_{8, 45} = 3.43$, $P < 0.005$). At the extremes, male #11 sired almost four times the female progeny sired by male #5. It is not clear if this variation was due to quantitative or qualitative differences in sperm produced by males, or to differences in female oviposition behavior. If the differences were due to sperm inadequacy of some sort, then we might expect the mates of males

Table III. Mean number (\pm SD) of male, female and total progeny, and percent female progeny produced in first and last hosts parasitized by females that parasitized either three (N = 28) or four (N = 23) hosts.

Mean (\pm SD)	Three Hosts Parasitized			Four Hosts Parasitized			Last Host					
	First Host	Last Host	First Host	First Host	Second Host	Third Host	Fourth Host	Fifth Host	Sixth Host	First Host	Second Host	Third Host
	♀	♂	Σ	♀	♂	Σ	♀	♂	Σ	♀	♂	Σ
Number	8.1	1.8	9.9	7.2	2.4	9.6	9.3	3.5	12.9	5.9	2.9	8.8
	(5.2)	(1.8)	(5.7)	(4.8)	(2.4)	(5.5)	(5.8)	(3.5)	(6.5)	(5.4)	(3.3)	(6.4)
Percent females	79.0			74.0			70.9			65.8		
	(23.3)			(25.6)			(25.5)			(35.1)		

Table IV. Mean number (\pm SD) of female and male progeny produced by females, grouped by the male to whom they were mated.

	Male Identification Number								
	4	5	6	10	11	12	13	18	20
Mean number	20.8	11.3	12.8	21.8	41.5	31.5	22.3	24.2	19.5
female progeny	(6.9)	(7.0)	(10.4)	(11.6)	(15.8)	(11.6)	(10.7)	(19.4)	(10.6)
Mean number	7.0	10.2	21.3	4.5	8.3	10.2	19.5	7.3	5.8
male progeny	(4.6)	(10.1)	(20.8)	(2.4)	(2.3)	(8.4)	(13.3)	(2.3)	(5.1)

that sired fewer females to produce more male progeny, i.e., that there be a significant inverse association between the average number of male and female progeny produced. There were significant differences in the mean number of male progeny produced by females (ANOVA, $F_{8, 45} = 2.25$, $P < 0.05$). However, although the expected inverse correlation between mean number of male and female progeny was present, it did not approach significance ($r = -0.29$, $t = 0.81$, $df = 8$, $P > 0.40$).

DISCUSSION

There is little evidence that sperm depletion is an important factor limiting production of female progeny in populations of *P. venustus*. First, males are more than capable of inseminating the usual ratio of female sibs that share a host with them (Fig. 1, Table I). Second, there was no evidence that males supplied less sperm to later mates than they did to earlier ones: The order in which females were inseminated did not influence the number of female progeny they produced (Table II). And third, in most instances females tended to produce as many female progeny in the last cell parasitized as in the first (but see below, Table III). Thus, this study joins others (e.g., Sekhar 1957, Gordh and DeBach 1976, Nadel and Luck 1985, Suzuki and Hiehata 1985, Dijkstra 1986, Rojas-Rousse, et al. 1988) in supporting the assumption of sex ratio theorists that male parasitoids are fully capable of inseminat-

ing their sisters over periods of time in which they are likely to encounter them under usual conditions.

In contrast, some females might have experienced at least temporary egg depletion. Females that parasitized four hosts (but not those that parasitized three hosts) produced significantly fewer progeny on the last host parasitized than on the first and this reduction was expressed in the female sex but not in the male (Table III). It is not clear why total progeny would decline if sperm was depleted; why would ovipositing females not simply replace fertilized eggs with unfertilized ones? An average male consumes roughly 66.7% of the resources consumed by an average female (Tepedino, unpublished); thus two fertilized eggs could be replaced by up to three unfertilized ones without affecting the fitness of the other progeny. The fact that females did not behave in this manner suggests that egg depletion is a more likely explanation for the reduction in progeny than sperm depletion. Only additional experiments will resolve this issue.

The theoretical and experimental work of Green et al (1982) showed that gregarious female parasitoids under LMC should produce the exact number of males necessary to inseminate their host-sharing sisters. Since males can inseminate four or more females without impairing their average fitness, one would expect female parasitoids to produce a sex ratio of about four females per male. Although populations of *P. venustus* sometimes display a sex ratio of more than three females to one male, and individual hosts in these populations often yield parasitoids whose sex ratio is even more female biased (Whitfield and Richards 1985, Tepedino, unpublished), the average sex ratio is 1.5–2.0 females per male. Thus, males are capable of inseminating at least twice the ratio of females that they are likely to encounter in an average host. Why then do female *P. venustus* not produce more female, and fewer male progeny, in each host?

Perhaps the answer to this question lies in the selection pressures that *P. venustus* has experienced since one of its two known hosts (Hobbs 1968), the alfalfa leafcutting bee, has become semi-domesticated. These host species normally occur at much lower densities in nature than they do in nest shelters in commercial alfalfa fields. With the semi-domestication of *M. rotundata*, the density of host and parasitoid have risen tremendously: hundreds of thousands of *M. rotundata* now typically nest in each

shelter. Unlike parasitoids emerging in natural populations, *P. venustus* in nesting shelters emerge in close proximity not just to siblings, but to numerous, synchronously emerging conspecifics as well, i.e., a situation approaching panmixis. More recently, the development of the "loose cell technique", in which leafcutting bee cells are ejected from their tunnels in the fall and stored in holding trays over winter, must thrust the breeding system even closer to panmixis because parasitoids now emerge synchronously from thousands of contiguous host cocoons in late spring/early summer shortly after incubation of bee cells begins. Under panmixis, female *P. venustus* that produce a sex ratio that is closer to unity would be at a selective advantage (Fisher 1958, Hamilton 1967). This is exactly the direction in which the sex ratio of *P. venustus* populations seems to have moved. The higher than expected percent of male *P. venustus* in the population may thus result from reduction in LMC due to the domestication of its host.

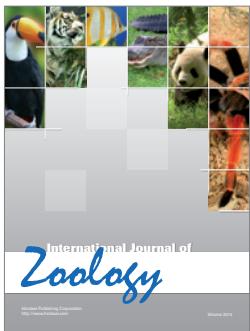
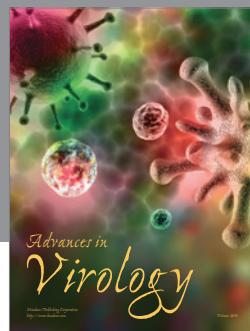
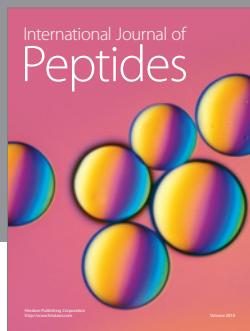
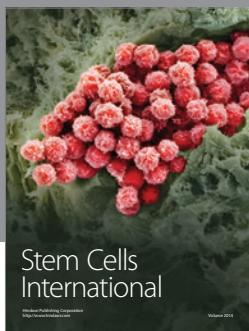
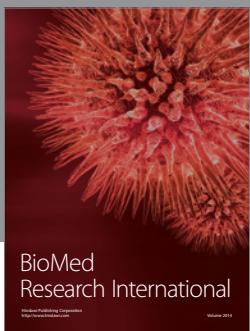
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