Mating Behavior of Platycotis vittata (Fabricius) (Homoptera: Membracidae)

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ABSTRACT: *Platycotis vittata* females mate up to five times in large field cages. Females that mate more than once have more viable eggs and surviving offspring than those that mate once. Electrophoretic data indicate that sperm displacement occurs with sperm from the last male fertilizing most or all eggs. The last mating occurs when ovarian development is incomplete and several weeks before oviposition. We suggest that continued female receptivity appears to be a mechanism which promotes matings with males that have demonstrated their longevity.

INTRODUCTION

Parental care of offspring is common in the Membracidae (Wood, 1974, 1976a,b, 1977, 1978, 1979). When parental investment in offspring is high, females should choose mates which contribute to the fitness of offspring (Trivers, 1972; Thornhill, 1979). For females which brood only one clutch, the consequences of a mating with a genetically less fit male are greater than for those producing several broods resulting from multiple matings.

In treehoppers, such as *Platycotis vittata* (Fabricius) and *Umbonia crassicornis* Amyot and Serville, longevity of females is critical to the survival of offspring. Nymphal survival is dependent on parent females making slits in the bark for feeding and females defending nymphs from predators (Wood, 1976a, b). Female *U. crassicornis* mate several days before eggs are deposited for about 1 hr (Wood, 1974). *Umbonia crassicornis* is a tropical and semitropical species living on evergreen legumes. Since there are no seasonal limitations on nutrient availability, it has continuous and overlapping generations throughout the year (Mead, 1962; Wood, 1974).

Here we examine the mating behavior of *Platycotis vittata,* the only North American relative of *Umbonia crassicornis.* The life history, host plants and geographical distribution of *P. vittata* differ in fundamental ways from *U. crassicornis,* and this suggests mating behavior may also differ. *Platycotis vittata* occurs in the highlands of Mexico and North America, using a number of species of oak (*Quercus*). In both Florida and Ohio, this species has two generations a year. In the spring (in Ohio), eggs are deposited by overwintering females before buds break. Nymphs molt to adults in early June with mating beginning in late June and July. Mating takes from 8-24 hr. Oviposition, however, does not occur until late August or early September (Wood, 1976b). Thus *P. vittata* differs from *U. crassicornis* in its 2-month delay between the onset of mating and oviposition in addition to its extremely long mating times. We present data to show that this prolonged delay between the initiation of mating and oviposition affects the mating behavior of *P. vittata.*

METHODS

Behavior.—During each of 3 consecutive summers (1980-1982), aggregations of nymphs were collected in the New Jersey Pine Barrens and reaggregated on white oak (*Quercus alba*) in large field cages (1.8m x 1.8m x 1.8m) located in Newark, Delaware. When nymphs molted to adults, they were tagged with color-coded numbered tags. A total of 341 females and 312 males were tagged. When precopulatory pairing commenced, daily observations were made every hour throughout the day (1980—7 AM to 9 PM; 1981-82—8 AM to 8 PM) until all males had died (32-51 consecutive days). For

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each hourly observation we recorded the tag numbers of all individuals engaged in precopulatory and mating pairs. We could then determine the age (in days), the duration (in hours) and number of times various behaviors occurred. To determine reproductive success of females, the number of eggs in each clutch, the number of eggs that hatched and survival of nymphs were recorded.

Electrophoresis.—*Platycotis* used for electrophoretic analysis were maintained frozen at – 70C until ½ hr prior to analysis. Insects were individually crushed in 45 μl of 2% 2-phenoxyethanol. Filter paper wicks (9 mm x 5 mm) were used to absorb the samples which were placed directly into the appropriate gel.

Two phosphoglucomutase (*Pgm,* EC 2.7.5.1) loci, one malate dehydrogenase (*Mdh,* EC 1.1.1.37) locus and one glutamate oxaloacetatetransaminase (*Got,* EC2.6.1.1) locus were polymorphic. *Pgm* was analyzed using continuous Tris-citrate, *p*H 8.0 gels (Selander *et al.,* 1971) while *Mdh* and *Got* were assayed in amine citrate gels, *p*H 6.1 (Clayton and Tretiak, 1972). Staining procedures for the three enzymes followed Selander *et al.* (1971). All gels were 15% starch (Sigma Chemical Co., St. Louis).

Banding pattern interpretation, identification of monomeric and dimeric proteins and homozygous and heterozygous individuals followed methods and assumptions discussed in recent papers (Manwell and Baker, 1970; Tracey *et al.,* 1975) and are not repeated here. Zones of activity on a gel which varied independently of other zones were numbered according to mobility; the fastest anodally migrating zone was designated locus one, the next two and so on. At a given locus the allele with the greatest anodal migration was designated *F*, the next *M* and the last, when it appeared, *S*.

RESULTS

Mating.—Precopulatory pairing and mating occurs in the morning and evening. Only seven of 157 precopulatory pairs and two of 72 matings observed in 1981 occurred between 12:00 noon and 4:00 PM. The mean temperature at which precopulatory pairs formed was 22.77 ± 6.16 C (SD) ($N = 157$) and for mating 24.26 ± 3.87 (SD) ($N = 72$). The time of day and temperature were not related for precopulatory pairing or for mating. Formation of precopulatory pairs by males is no guarantee of mating success; only three of 245 precopulatory pairs observed in 1981 resulted in matings. This suggests that females may be selective in their choice of partners.

We observed 188 females mating in 1981-1982. Females were divided into five groups based on the number of matings (Table 1). Neither the age at which females mated first nor the length of mating differed among the five groups. No differences were found in the age or length of mating in females that mate two, three or four times. Length of mating increased with subsequent matings for those females which mated three times ($R^2 = 0.13$, $r = 0.36$, $df = 58$, $P < 0.01$, $Y = 1.62 + 4.28\ X^1$). Both age and length of mating were related for females ($R^2 = 0.13$, $r = 0.36$, $P < 0.01$, $df = 58$, $Y = 7.84 + 0.372\ X^1$) that mate three times, as well as females that mated only once ($R^2 = 0.148$, $r = .38$, $df = 119$, $P < 0.01$).

Ovarian development.—Females collected when they were 14, 26, 36 and 44 days old showed no ovarian development. The 44-day-old females showed little or no egg development but their spermathecae were quite large. Thus, when most of the females had already mated and approximately 25 days before oviposition, the ovaries do not contain mature eggs.

Effect of multiple mating on female fitness.—We divided 42 females into two groups on the basis of whether they mated once or more than once. The length of the last mating and the number of eggs per clutch did not differ between the two groups (Table 2). Females that mated more than once spent more total hours mating, had a shorter number of days between the last mating and oviposition, and had more eggs hatch than

TABLE 1.—Comparison of mating length (in hours) and the age (days) of *Platycotis vittata* females that mated one or more times. Means are expressed with their standard deviations. ANOVA was used to compare within columns

		Number of matings				
	N	1	2	3	4	5
1. Female age	121	33.36 ± 8.99				
length (hr)		6.70 ± 10.74				
2. Female age	38	29.50 ± 6.80	37.42 ± 8.11			
length (hr)		5.61 ± 8.13	10.08 ± 12.15			
3. Female age	20	32.70 ± 7.97	40.40 ± 8.28	46.00 ± 7.72		
length (hr)		3.10 ± 2.34	6.05 ± 9.14	11.65 ± 13.03		
4. Female age	7	27.57 ± 4.93	32.57 ± 4.54	41.86 ± 8.44	46.57 ± 8.44	
length (hr)		4.07 ± 4.25	5.71 ± 5.31	9.43 ± 12.32	20.57 ± 28.41	
5. Female age	2	31.00 ± 8.49	42.00 ± 2.83	44.50 ± 2.12	49.00 ± 5.66	52.00 ± 7.07
length (hr)		2.00 ± 1.41	2.50 ± 0.71	1.00 ± 0.00	25.00 ± 1.41	29.50 ± 9.19
F Value (age)		2.132	1.986	0.714	0.140	
P		0.0786	0.1251	0.4992	0.7196	
F Value (length)		0.808	0.974	0.674	0.044	
P		0.5215	0.4109	0.5185	0.8397	

those that mated only once. We then ran a stepwise regression of number of eggs in a clutch or arc sine of percent egg hatch against the following variables: length of last mating, age female mated last, cumulative mating hours, number of days from last mating to oviposition and if they had one or more matings. None of these variables was important in determining the number of eggs in a clutch. The only variable which affected egg hatch was whether females mate once or more than once ($F = 4.88$, $P = 0.033$, $df = 40$). Since neither the number of days between last mating and oviposition nor the age of the female at last mating was important in determining egg hatch, fertilization of eggs probably occurs after mating. Since ovarian development is delayed in this species, we might expect that females which were mated at older ages, with more fully developed eggs, would have more viable eggs if fertilization occurred shortly after mating. In terms of female fitness, neither the duration of the last mating nor the cumulative hours spent mating is important in egg hatch. These factors should be related if females were receiving some nutrient from the male which was necessary to produce viable eggs.

Apparently no benefits accrue from mating more than twice. Females which mated two or more times did not differ in cumulative mating time, days between last mating and oviposition, number of eggs in clutch, and in the percent egg hatch.

Electrophoretic analysis.—Data from 15 field-inseminated females and their offspring substantiated our belief that multiple matings occur under natural conditions. Thirteen of the 15 sets of offspring possessed genotypes that were significantly different from expected when it was assumed that the alleles could be used as neutral markers and the female parent of each set mated with only one male (Table 3).

Deviations from expected values in most broods appear to result from a few aberrant individuals (Table 3). For example, of female #3's offspring, the seven *F/M Mdh*-2 individuals necessitate the *F/M* male parental genotype. If we assume that the female mated with two males, one *M/M* and one *F/F* (or *F/M*) at *Mdh*-2, and further assume that the latter male fertilized few eggs, the significant deviation from expected would be eliminated. If the two males' *Pgm*-1 genotypes were *F/F* and *F/M*, respectively, the expected genotypic ratios of the offspring would be in accord with observed values.

Genotypes of 11 of the 13 sets of offspring would be in accord with expected ratios if we consider that two males mated with each female and one male fertilized most of the eggs. One of the combinations needed to explain *Pgm*-1 ratios for progeny in set #19 would still be significantly different from expected ($P = 0.05$). Results obtained in set #5 for *Pgm*-1 cannot be reconciled with multiple mating patterns; differential survival may be involved in this group (43% of the eggs laid by this female did not hatch).

Field-cage matings indicate that female *Platycotis vittata* can mate at least five times. However, electrophoretic data from field-collected females and their young are best interpreted by assuming fertilization by two males. Both sets of data can be reconciled if we assume that the sperm from the last male can exclude most or all of sperm deposited

TABLE 2.—Comparison of *Platycotis vittata* females that mated once with those mating two or more times. Means are expressed with their standard deviations

	Single (N = 22)	Multiple (N = 20)	t	P
Length (hr) of last mating	9.45 ± 10.51	14.15 ± 12.78	-1.31	0.200
Age of female at last mating	33.73 ± 10.28	44.40 ± 10.15	-3.38	0.002
Cumulative mating time (hr)	9.45 ± 10.51	24.80 ± 16.65	-3.61	0.001
Day from mating to oviposition	39.09 ± 11.68	28.70 ± 10.96	2.96	0.005
Number of eggs	38.86 ± 12.84	44.00 ± 10.91	-1.39	0.172
Percent egg hatch (arc sine)	48.03 ± 20.58	60.80 ± 16.37	-2.21	0.033

TABLE 3.—Parent-offspring combinations for the polymorphic loci of 15 field-inseminated female *Platycotis vittata*. Putative male parental genotype was deduced from female-offspring genotypes. Chi-square values are calculated for the single mating hypothesis

Parent female	Locus	Female genotype	No. offspring of type						Male genotype	Single mating
			F/F	F/M	F/S	M/M	M/S	S/S		
1	*Mdh-2*	*M/M*				18			*M/M*	
	Pgm-1	*M/M*				18			*M/M*	
	Pgm-4	*M/M*		4		14			*F/M*	5.6*
	Got-1	*M/M*		7		11			*F/M*	n.s.
2	*Mdh-2*	*M/M*				18			*M/M*	
	Pgm-1	*M/M*				18			*M/M*	
	Pgm-4	*M/M*				18			*M/M*	
3	*Mdh-2*	*M/M*		7		32			*F/M*	16.0**
	Pgm-1	*F/M*	21	16		2			*F/M*	19.8**
4	*Mdh-2*	*M/M*				37			*M/M*	
	Pgm-1	*F/F*	32	7					*F/M*	16.0**
	Pgm-4	*F/M*	10	4		7			*F/M*	8.9**
	Got-1	*M/M*		25		14			*F/M*	n.s.
5	*Mdh-2*	*M/M*				39			*M/M*	
	Pgm-1	*M/S*			2		30	7	*F/S*	46.5**
	Got-1	*M/M*		19		14	6		***	****
9	*Mdh-2*	*M/M*				18			*M/M*	
	Pgm-1	*M/M*				18			*M/M*	
	Pgm-4	*M/M*		5		13			*F/M*	n.s.
11	*Mdh-2*	*M/M*				18			*M/M*	
	Pgm-1	*M/S*		1		7	10		*F/M*	12.0**
	Got-1	*M/M*		3		15			*F/M*	8.0**
12	*Mdh-2*	*M/M*		1		17			*F/M*	14.2**
	Pgm-1	*F/M*	4	11		3			*F/M*	n.s.
14	*Mdh-2*	*M/M*		6		33			*F/M*	18.7**
	Pgm-1	*F/F*	34	5					*F/M*	22.8**
	Pgm-4	*M/M*				21			*M/M*	
	Got-1	*F/M*	1	8		10			*F/M*	9.0**

TABLE 3.—continuing

Parent female	Locus	Female genotype	No. offspring of type						Male genotype	Single mating
			F/F	F/M	F/S	M/M	M/S	S/S		
15	*Mdh-2*	*M/M*				39			*M/M*	
	Pgm-1	*F/M*	10	6	7	13	3		***	****
	Pgm-4	*M/M*		3		36			*F/M*	27.9**
	Got-1	*M/M*				39			*M/M*	
16	*Mdh-2*	*M/M*		4		14			*F/M*	5.6*
	Pgm-1	*F/M*	6	3		6	3		***	****
	Pgm-4	*M/M*		4		14			*F/M*	5.6*
	Got-1	*M/M*		3		15			*F/M*	8.0**
17	*Mdh-2*	*M/M*				18			*M/M*	
	Pgm-1	*F/M*	9	5	2				*F/S*	9.7**
	Got-1	*M/M*				18			*M/M*	
18	*Mdh-2*	*M/M*		3		15			*F/M*	8.0**
	Pgm-1	*F/F*	16	2					*F/M*	12.5**
19	*Mdh-2*	*F/M*		7		11			*M/M*	n.s.
	Pgm-1	*F/M*	2	3		13			*F/M*	20.1**
	Got-1	*M/M*				18			*M/M*	
20	*Mdh-2*	*M/M*		7		11			*F/M*	n.s.
	Pgm-1	*M/M*				18			*M/M*	
	Pgm-4	*M/M*		3		15			*F/M*	8.0**
	Got-1	*F/F*	8	9					*F/M*	n.s.

*P<0.05
**P<0.01
***indicates impossibility of single mating hypothesis

during previous matings. Three laboratory multiple-mated females and their young were electrophoretically analyzed. Although two (BY41 and G07) had mated with two males and the third (BY30) mated with three males, the ratios of all progeny genotypes were consistent with that expected through fertilization of eggs by a single male (Table 4).

DISCUSSION

Prolonged ovarian development and lack of males at the time of oviposition are constraints which affect the mating system of *Platycotis vittata*. Females do not have mature ovaries at the age of first mating and do not deposit their eggs for approximately 30 days after their first mating. In contrast, in *Umbonia crassicornis* oviposition occurs shortly after mating, suggesting ovarian development is complete (Wood, 1974). Female *P. vittata* that mate early must store sperm for several weeks before oviposition, which may affect sperm viability. Females must mate early because male mortality is very high with few males alive at the time of oviposition. Thus females cannot predict the availability of males at the time ovarian development is complete.

Females may mate initially to secure sperm as insurance against the unpredictability of males at the time of oviposition. As females remate with older males they appear to refill their spermathecae with sperm from the last male. Thus females that remate and replenish sperm throughout the season are mating with progressively older males. The greater viability of eggs from females that remate implies that they have a selective advantage over females that mated once, early in the season. Electrophoretic data imply displacement of sperm with the last male fertilizing the majority of eggs (Gromko and Pyle, 1978). In *Drosophila melanogaster* fertility is greater in multiple-mated females and appears to be the result of fresher sperm used for fertilization (Gromko and Pyle, 1978). In *Platycotis vittata* the duration between mating and oviposition was not related to egg hatch, suggesting sperm viability is not a factor. Thus, continual receptivity appears to be a mechanism which promotes matings with males that have demonstrated their longevity.

Since *Platycotis vittata* females may remate, there is no assurance of paternity and selection should favor males that protect sperm from displacement. Mating duration increases in the second to fifth mating of females, suggesting males are attempting to assure paternity. Although females potentially could remate, the number of available males decreases dramatically by the end of July, reducing the probability of subsequent matings. In other insects, mating duration may not necessarily be an indication of amount of sperm transfer but rather reflect a male's efforts to assure paternity (Smith, 1979; Parker, 1970) or transfer other materials essential for ovarian development (Gwynne, 1981; Friedel and Gillott, 1977; Boggs and Gilbert, 1979).

Sibling mating of *Platycotis vittata* may be reduced by a differential dispersal from aggregations and host plants. If *P. vittata* males disperse from hosts and females stay, this would place added selective pressures on females to mate early. If females wait to mate when ovarian development is complete, they are dependent upon males locating them as the absolute number of males in the population is decreasing. Females could leave hosts in search of males but we found no evidence of such movements in cages.

The question of why *Platycotis vittata* females remate seems to reside with host plant interactions. The life history of *P. vittata* parallels the physiology of its *Quercus* hosts. Females emerge in the spring from the leaf litter and deposit eggs prior to bud break. *Platycotis vittata* eggs hatch when buds break, at a time when nymphs can use the upward flow of nutrients (Longman and Coutts, 1974). In the latter part of June after nymphs have molted to adults, leaves develop increased tannin, and nutrient content of the phloem is reduced (Longman and Coutts, 1974). Females begin to mate in June and continue through early August. The low nutrient content of phloem may slow ovarian development. Oviposition occurs in late August with eggs hatching in early to mid-September when leaves begin senescence. Oviposition appears to be timed to provide nymphs with nutrients being translocated from leaves to the roots.

TABLE 4.—Parent-offspring combinations for the polymorphic loci of three field-cage multiple-inseminated *Platycotis vittata*. Putative male parental genotype was deduced from female-offspring genotypes. Chi-square values are calculated for the single mating hypothesis

Parent female	Actual # of matings	Female		No. offspring of type						Male genotype	Single mating
		Locus	genotype	F/F	F/M	F/S	M/M	M/S	S/S		
BY41	2	*Mdh-2*	*M/M*				18			*M/M*	
		Pgm-1	*F/M*	4	4		5			*F/M*	n.s.
		Pgm-4	*M/M*				13			*M/M*	
GO7	2	*Mdh-2*	*M/M*		8		9			*F/M*	n.s.
		Pgm-1	*F/F*		10	7				*M/S*	n.s.
		Pgm-4	*F/M*		12		5			*M/M*	n.s.
BY30	3	*Mdh-2*	*M/M*		8		10			*F/M*	n.s.
		Pgm-1	*F/M*	3	7		8			*F/M*	n.s.
		Got-1	*M/M*				18			*M/M*	

The mating system of *Platycotis vittata* appears to be the result of selective trade-offs imposed by the availability of nutrients to raise offspring. Females should mate with long-lived males, but high male mortality and dispersal promotes early mating and sperm storage. Continued female receptivity and sperm displacement permit females to mate with older males but also decrease the storage time for sperm.

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