REINSEMINATION OF PAROUS *CULEX TARSALIS* FEMALES

WILLIAM K. REISEN, BRENDA G. EVANS AND MARTHA E. BOCK

Arbovirus Field Station, Department of Biomedical and Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA 94720

ABSTRACT. Remating by parous *Culex tarsalis* females was studied in laboratory cages using the recessive, autosomal eye pigment mutant, carmine. The frequency of reinsemination in reciprocal crosses was low (0.028 and 0.010) and did not increase with successive gonotrophic cycles, contrasting previous observations using a radioactive tracer method.

INTRODUCTION

Pioneering experiments by Craig (1967) revealed that female mosquitoes are rendered monogamous by matrone, a hormone transferred with male semen during mating. Multiple insemination was reported infrequently for nulliparous *Culex tarsalis* Coq. females using radiosterilized males (Zalom et al. 1981), but was not detected using genetic markers in laboratory cage competition experiments (Asman 1975a, Ainsley and Asman 1980). Delayed remating among nullipars was not detected by Zalom et al. (1981). More recently, Young and Downe (1983) observed a high frequency of remating among biparous females using a radiotracer technique. These findings supported studies with *Aedes aegypti* (L.) by Williams and Berger (1980) and Young and Downe (1982) who similarly observed a high incidence of remating among multiparous females. The contrasting present account describes an infrequent occurrence of remating among parous *Cx. tarsalis* females using a genetic detection system.

MATERIALS AND METHODS

STRAINS: 1. *car:* Carmine eye is a recessive, autosomal eye color pigment mutant with good penetration in all immature stages, but reverts to wild-type in mature adults (Asman 1975b). Adults with *car* mated competitively against laboratory-adapted wild-type adults in insectary cage competition experiments (Ainsley and Asman 1980).

2. *Br*80: Breckenridge 1980 is a laboratory-adapted, wild-type colony (Reisen et al. 1982).

MOSQUITO HANDLING. Young females (1–3 days old) were allowed to cohabit 1 ft^3 (0.03 m^3) cages with mature (3–8 days old), virgin males for a 3–6 day period after emergence. Females were then offered a restrained chick as a blood meal source and when gravid, were isolated in 6 dram vials for oviposition. Ovipositing females were released into a second cage without examining their egg rafts for hatch or the phenotype of their progeny. A second group of males was then added to the second cage for > 2 days, after which the females were refed and isolated for a second oviposition. The duration of cohabitation was contingent upon the synchrony of oviposition. Progeny from cycle 2 were reared as single families to second or third instar and their eyes examined for pigmentation. Families with both wild and carmine eye phenotypes indicated a reinsemination and were reared to pupation to confirm presumptive larval determinations. New males were added and the procedure repeated during each subsequent gonotrophic cycle until too few females survived to be meaningful.

CROSSES. Reciprocal crosses were performed in 2 experiments: Exp. 1: *car* ♀ were crossed with sibling *car* ♂ for gonotrophic cycle 1 and then crossed with *Br80* ♂ for subsequent cycles. Exp. 2: *car* ♀ were crossed with *Br80* ♂ for gonotrophic cycle 1 and then with *car* ♂ for subsequent gonotrophic cycles. In both experiments the presence of phenotypically wild (+/*car*) and carmine (*car*/*car*) eye immatures in the same family indicated that the female was reinseminated by either a *Br80* or *car* ♂. More than 1000 pupae from both the *car* and *Br80* colonies were examined for contamination just before and just after experimentation with negative findings.

RESULTS AND DISCUSSION

The proportion of 1-parous females remating, i.e., ovipositing mixed families with both *car* and + phenotypes, did not differ significantly ($P > 0.05$) between experiments (Exp. 1 = 0.028, Exp. 2 = 0.010, $\chi^2 = 1.3$, Table 1). The failure to detect remating during gonotrophic cycles 3 to 5 was attributed to the death of the females that had remated during cycle 2. Some *car* ♀ did not mate during gonotrophic cycle 1 and produced all wild or all *car* families during subsequent cycles in Exp. 1 or 2 after mating with a *Br80* or *car* ♂, respectively. The proportion of *Cx. tarsalis* remating in the present study was much lower than observed by Young and Downe (1983) who reported that up to 66% of biparous females became radioactive (i.e., were

Table 1. Remating in colonized *Culex tarsalis* detected by a genetic system.

Gonotrophic cycle	No. ♀	No. ♂	No. families scored car	wild	mixed	total
Exp. 1: *car* ♀ × *car* ♂ for cycle 1, then *Br80* ♂ for cycles 2 and 3.						
1	1200	1200	—	—	—	—
2	260	ca 1800	98	7	3	108
3	60	120	18	2	0	20
Exp. 2: *car* ♀ × *Br80* ♂ for cycle 1, then *car* ♂ for cycles 2 to 5.						
1	485	485	—	—	—	—
2	324	400	5	186	2	193
3	130	300	2	64	0	66
4	55	100	1	39	0	40
5	38	50	0	25	0	25

remated) upon exposure to radiolabelled males at a 1 ♂:3 ♀ ratio.

Marked differences in the frequency of remating reported for 2 strains of *Cx. tarsalis* in the same size cages using different detection methods was unanticipated and difficult to interpret. Possibly, remating males transfer ejaculate without successfully transferring sperm to the female spermathecae. Thus, ejaculation renders the female radioactive without altering the genotype of her progeny. The increased fecundity among multiparous females documented by Young and Downe (1983) may have resulted from the transference of matrone during remating; however, a mechanism which would allow semen transfer to the bursa copulatrix without the addition of sperm to the spermathecae was difficult to conceptualize.

Alternatively, *Cx. tarsalis* from Kern Co., California, and Saskatoon, Saskatchewan, have evolved markedly different reproductive strategies. Perhaps at cooler latitudes where adults survive longer, remating to renew fertility has a greater advantage than at warmer latitudes where relatively few adults survive more than one gonotrophic cycle (Reisen et al. 1983). The extrapolation of these laboratory findings to nature would seem critical in assessing the importance of remating in the reproductive biology and population dynamics of *Cx. tarsalis.*

In the present experiment, families with mixed progeny exhibited pupal phenotypic ratios which approached 1 *car:* 1 wild (Table 2). Thus, the male mating second was able to contribute almost equal amounts of sperm to the second family as the male which mated first. In contrast, Mahmood and Reisen (1980) found that 3 of 5 nulliparous females remating after a 3 day postinsemination period produced progeny mostly originating from the initial insemination. Perhaps sperm losses incurred during oviposition provided sufficient room for the addition of an equal volume of sperm during a second insemination.

Table 2. Pupal phenotypes of mixed families.

Exp.	Family	Pupal phenotype *car*	wild	total	χ^2 for 1:1
1	1	5	9	14	1.14 ns
	2	1	16	17	13.23 ($P<0.01$)
	3	6	4	10	0.40 ns
2	1	79	82	161	0.06 ns
	2	43	39	82	0.20 ns
	Total	134	150	284	0.90 ns

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References Cited

Ainsley, R. W. and S. M. Asman. 1980. Genetic fitness of the mutant, carmine eye, in *Culex tarsalis* in the laboratory. Mosq. News 40:221–224.

Asman, S. M. 1975a. Observations of mating behavior in *Culex tarsalis.* Ann. Entomol. Soc. Am. 68:777–778.

Asman, S. M. 1975b. The genetics of two new eye color mutants in *Culex tarsalis.* J. Hered. 66:297–300.

Craig, Jr., G. B. 1967. Mosquitoes: female monogamy induced by male accessory gland substance. Science 156:1499–1501.

Mahmood, F. M. and W. K. Reisen. 1980. Studies on multiple mating in *Anopheles culicifacies* Giles. Entomol. Exp. Appl. 27:69–77.

Reisen, W. K., M. M. Milby, S. M. Asman, M. E. Bock, R. P. Meyer, P. T. McDonald and W. C. Reeves. 1982. Attempted suppression of a semi-isolated *Culex tarsalis* population by the release of irradiated males: a second experiment using males from a recently colonized strain. Mosq. News 42:565–575.

Reisen, W. K., M. M. Milby, W. C. Reeves, R. P. Meyer and M. E. Bock. 1983. Population ecology of *Culex tarsalis* (Diptera: Culicidae) in a foothill environment in Kern County, California: temporal changes in female relative abundance, reproductive status and survivorship. Ann. Entomol. Soc. Am. 76:800–808.

Williams, R. W. and A. Berger. 1980. The relation of

female polygamy to gonotrophic activity in the Rock strain of *Aedes aegypti.* Mosq. News 40:597–604.
Young, A. D. M. and A. E. R. Downe. 1982. Renewal of sexual receptivity in mated female mosquitoes, *Aedes aegypti.* Physiol. Entomol. 7:467–471.
Young, A. D. M. and A. E. R. Downe. 1983. Influence of mating on sexual receptivity and oviposition in the mosquito, *Culex tarsalis.* Physiol. Entomol. 8:213–217.
Zalom, F. G., S. M. Asman and J. E. Fields. 1981. Tests for multiple insemination of females involving irradiated and un-irradiated male *Culex tarsalis* (Diptera: Culicidae) Mosq. News 41:154–6.