COMPARISON OF REPRODUCTIVE CHARACTERISTICS OF LABORATORY AND FIELD-COLLECTED *CULEX TARSALIS* IN LABORATORY CAGES[1]

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ABSTRACT. The fecundity, insemination status, oviposition rate and fertility of females from crosses with laboratory colony and/or field-collected populations of *Culex tarsalis* were determined. The insemination rate was highest for inbred laboratory females (96%), less for laboratory females outcrossed to field-collected males (78%), still less for field-collected females outcrossed to laboratory males (47%), and least for inbred field-collected females (28%). The oviposition rate for inseminated field-collected females was 73% compared with 98% for laboratory females. While 77% of the eggs developed in ovaries of inbred laboratory females ultimately hatched, only 12% of those developed by inbred field-collected females hatched. Intermediate percentages of developed eggs hatched in the 2 types of outcrossed females.

Success in the colonization of mosquitoes varies greatly from species to species. Even within a species, some field-collected populations readily colonize while others cannot be established and maintained despite considerable effort. The problems of colonization frequently are related to deficiencies in mating, oviposition and egg hatch.

The success of efforts to cross field-collected and laboratory colony populations is unpredictable. In *Culex nigripalpus*, field males can be successfully crossed with laboratory females while field females will not cross with laboratory males (Haeger and O'Meara 1970). Female *Aedes taeniorhynchus* from field-collected eggs are much less likely to mate with laboratory males in laboratory cages than are male adults from those eggs likely to mate with laboratory females (O'Meara and Evans 1974).

In 1977, we established a program to monitor the success of a field release of genetically-altered *Cx. tarsalis*. In this program males derived from field-collected pupae were crossed to genetically-marked laboratory females. Matings were done either in cylindrical 17 cm × 17 cm cartons or in colony cages, 60 cm on each side. These crosses produced few egg rafts and a high proportion were classified as low hatch rafts. It was later demonstrated that the males reared from

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field-collected pupae were not carrying the released genetic mechanism and their mating should have produced almost all high hatch egg rafts (Asman et al. 1979). During the same experiment field-mated females were collected, and almost all of these produced normal-hatch egg rafts. When field-collected males (reared from pupae or collected from shelters) were crossed in colony cages with laboratory colony females, less than 50% of their egg rafts hatched compared with 98% for rafts deposited by inbred laboratory colony females. Of the hatching rafts, approximately 50% from the laboratory × field outcross had low hatch while only 5% from the inbred colony had low hatch.

These findings led to the present studies of the mating, oviposition and hatching success when field-collected and laboratory mosquitoes were inbred or outcrossed with each other. Simultaneous experiments conducted outdoors in walk-in and smaller colony cages indicated that oviposition was affected by cage size, and that when field-collected females were confined in outdoor cages they frequently retained eggs instead of ovipositing (McDonald et al. 1978). The present experiments investigated the fecundity, insemination, oviposition and egg-hatching history of individual females under standard laboratory conditions of temperature, photoperiod, cage size and sex ratios used in crosses. The objective was to determine the types and extent of problems associated with laboratory colonization of a field-collected population.

MATERIALS AND METHODS

Two sources of mosquitoes were used: KL, a vigorous laboratory colony established 6 years previously from Knights Landing, Yolo County, California and PWW adults derived from pupae collected at Poso West, Kern County, California in late September, 1977. The experiment was conducted in an insectary at 26 ± 2°C with a daily 14 hr period of light supplemented with 2 half-hour periods of reduced light to simulate dawn and dusk.

Four laboratory populations of newly-emerged virgin mosquitoes were established in cages (60 cm × 60 cm × 60 cm) as follows: 1) 200 KL ♀ ♀ × 200 KL ♂ ♂, 2) 163 KL ♀ ♀ × 163 PWW ♂ ♂, 3) 200 PWW ♀♀ × 200 KL ♂ ♂, 4) 200 PWW ♀♀ × 200 PWW ♂ ♂ Mosquitoes were allowed to feed on a 10% sucrose solution but no oviposition site was provided for at least 4 days. Earlier studies by Asman (1975) suggested this was an adequate period for mating. When females were 3–5 days old a restrained chick was offered as a blood-meal source. After 1.5 days 100 blood-engorged females were taken from each group and transferred individually to numbered vials where they were provided with both water and a sugar solution. Vials were examined for egg rafts daily, and rafts were removed 3 days after oviposition. The numbers of hatched and unhatched eggs in each raft were determined. Those unhatched eggs which had eye spots were classified as embryonated and those without as unembryonated. At the end of a 6–7 day oviposition period the females were frozen. At a later date dissections were done to determine the numbers of mature (Christopher stage V) egg follicles in the ovaries and the presence or absence of sperm in the spermathecae. The number of inseminated females for each group was calculated as the number producing hatching rafts plus any others scored positive for sperm in the spermathacae. The insemination rate was the number of inseminated females divided by the number of females that developed eggs. The oviposition rate for inseminated females was calculated as the number of inseminated females that oviposited divided by the total number of inseminated females in the group.

RESULTS

Of the 400 females, 8 that had not oviposited or developed mature follicles (Table 1) were eliminated from further

analysis. Three females had oviposited fewer than 10 eggs (total = 9 eggs) and were classified as non-ovipositing. Twenty egg rafts had fewer than 10 eggs hatched (total = 44 eggs), and none of these had more than 5% of the total eggs hatched. These 20 rafts were classified as non-hatching.

The rates of egg development (fecundity), oviposition and hatching (fertility) were determined for each of the 4 population groups (Table 1). The mean number of eggs developed varied between each group, but the KL females consistently developed more eggs per individual than the PWW females. Nearly all inbred KL females oviposited, and the outcrossed KL females did almost as well (Table 1). In contrast, less than 50% of PWW females oviposited. The inbred KL females which oviposited retained an average of 1 egg (186 developed—185 oviposited). The outcrossed KL females retained an average of 6 eggs, the outcrossed PWW females 6 eggs, and the inbred PWW females 22 eggs.

The greatest number of hatching rafts was produced by the inbred KL cross (Table 1). The outcrossed KL females were less successful. KL females gave more hatching rafts than PWW females regardless of the male in the cross. More outcrossed PWW females produced hatching rafts than inbred PWW females. The females that deposited hatching rafts rarely retained developed eggs except that the inbred PWW females retained an average of 16 eggs per female. The percent hatching was essentially uniform from cross to cross (Table 1).

The insemination determinations for females in the several categories are shown in Table 2. The females were classified into 3 categories: 1) with hatching rafts, 2) oviposited but eggs did not hatch, and 3) ovaries developed but eggs retained as mature follicles, without oviposition. As expected, almost all females that oviposited hatching rafts were classified as inseminated based on spermathecal examination. Only 19% of the females that oviposited non-hatching rafts were classified as inseminated by dissection. Of the females that developed ovaries but did not oviposit 21% were classified as inseminated. Lack of insemination was closely correlated to no hatch and retention of eggs.

The 2 highest insemination rates involved KL females (Table 2). KL males inseminated more effectively than PWW males. The female type was the strongest determinant for insemination.

When oviposition rates were determined for inseminated females, the inbred and outcrossed KL females had simi-

Table 1. Reproductive characteristics of crosses involving laboratory (KL) and/or field (PWW) *Culex tarsalis*.

Cross ♀♀ x ♂♂	No. engorged ♀ ♀ sampled	♀ ♀ developing eggs		♀ ♀ ovipositing		
		No.	Eggs developed/♀ ($\bar{x}$ ± SD)	No.	Eggs developed/♀ ($\bar{x}$ ± SD)	Eggs oviposited/♀ ($\bar{x}$ ± SD)
KLxKL	100	98	186 ± 50	95	186 ±	185 ± 51
KLxPWW	100	98	201 ± 57	86	206 ± 57	200 ± 62
PWWxKL	100	96	150 ± 68	47	144 ± 73	138 ± 76
PWWxPWW	100	100	154 ± 52	45	159 ± 58	137 ± 69

Cross ♀♀x♂♂	♀ ♀ with hatching rafts				
	No.	Eggs developed/♀ ($\bar{x}$ ± SD)	Eggs oviposited/♀ ($\bar{x}$ ± SD)	Eggs hatched/♀ ($\bar{x}$ ± SD)	Percent hatch
KLxKL	90	187 ± 51	186 ± 51	156 ± 65	84
KLxPWW	64	215 ± 55	212 ± 57	176 ± 72	83
PWWxKL	33	144 ± 79	143 ± 79	120 ± 76	84
PWWxPWW	18	144 ± 68	128 ± 75	106 ± 78	83

Table 2. Insemination and oviposition rates of 3 classes of females from crosses involving laboratory (KL) and/or field (PWW) *Culex tarsalis*.

Cross ♀ ♀ x ♂ ♂	No. ♀♀ with hatching rafts scored as		No. ♀♀ with non-hatching rafts scored as		No. ♀♀ retaining all eggs scored as		Insemination** % ± SE	Oviposition*** % ± SE
	S+*	S−*	S+	S−	S+	S−		
KLxKL	86	4	2	3	2	1	96 ± 2	98 ± 1
KLxPWW	64	0	9	13	3	9	78 ± 4	96 ± 2
PWWxKL	29	4	0	14	12	37	47 ± 5	73 ± 7
PWWxPWW	16	2	2	25	8	47	28 ± 4	71 ± 9
Totals	195	10	13	55	25	94		
% of class	95	5	19	81	21	79		

* S+ sperm present, S− sperm absent in spermathecae.
** S-females with hatching rafts included.
*** For inseminated females.

lar high oviposition rates (Table 2). The PWW females, either inbred or outcrossed, had similar oviposition rates but were considerably lower than those of KL females.

Fifty-five uninseminated females deposited non-hatching rafts, and all but 3 of these occurred in crosses involving 1 or both PWW parents (Table 2). Thirteen inseminated females produced non-hatching rafts, and 9 of these came from outcrossed KL females (Table 2).

The percent of developed eggs which hatched was calculated for each cross as a partial indicator of reproductive success (Table 3). In each cross almost all embryonated eggs hatched. The well-adapted laboratory colony had the greatest success, 77% compared with 12% for the field-collected population. The 2 outcrosses showed intermediate levels of success.

DISCUSSION

The comparison of the crosses between laboratory females and field-collected or laboratory males (Table 2) showed that the field-collected males were less adept at insemination than laboratory colony males under laboratory conditions. This explains part of the previous difficulties experienced with the laboratory female × field male cross (Asman et al. 1979). However, the past observations of high frequency of low hatch rafts were not made in this experiment, possibly because a sex ratio of 1 male to 1 female was used in these experiments and a 1 male to 5 female ratio was used in the field monitoring experiment.

In mosquitoes, matrone is transferred during insemination and later triggers oviposition (Hiss and Fuchs 1972). Thus the normal sequence, illustrated by the KL inbred cross (Table 2) is insemination followed by oviposition. Deposition of sterile rafts by 55 uninseminated females was unexpected. These could have represented females with incomplete insemination which occurs when males mate that have partially depleted their semen

Table 3. Egg development, oviposition, embryonation and hatch from crosses involving laboratory (KL) and/or field (PWW) *Culex tarsalis*.

Cross ♀ ♀x♂ ♂	No. eggs				Embryonated eggs hatched % ± SE	Developed eggs hatched % ± SE
	Developed	Laid	Embryonated	Hatched		
KLxKL	18,213	17,540	14,603	14,016	96 ± 0.2	77 ± 0.3
KLxPWW	19,740	17,218	11,697	11,256	96 ± 0.2	57 ± 0.4
PWWxKL	14,407	6,491	4,072	3,971	98 ± 0.2	28 ± 0.4
PWWxPWW	15,425	6,142	1,970	1,914	97 ± 0.4	12 ± 0.3

(Gwadz and Craig 1970, Hauserman and Nijhout 1975); or these females may have been improperly rated as uninseminated. Even so, it is rare to encounter no hatch rafts from inseminated females except in cases of genetic incompatibility or in the presence of a lethal agent.

Laboratory tests conducted after these experiments indicated that PWW field females confined in vials oviposit more readily than those in colony cages (unpublished data). It would seem then that the lower oviposition rate of PWW females in the experiments reported here was due to the general process of colonization rather than the use of vials. The insemination rate for PWW females and the oviposition rate for mated PWW females both were less than the corresponding rates for KL females (Table 2), and the product of these 2 factors accounts for their substantial loss of reproductive ability in laboratory cages. From these findings there can be little doubt that colonization acts as a major selective force. It is important to determine if such selection can be avoided by outcrossing at initial colonization, use of large cages, or keeping colonies outdoors in more natural environmental conditions.

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