STUDIES ON AUTOGENY IN *CULEX TARSALIS*: 2. SIMULATED DIAPAUSE INDUCTION AND TERMINATION IN GENETICALLY AUTOGENOUS FEMALES[1]

WILLIAM K. REISEN

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

ABSTRACT. Exposure to simulated "mid-winter" contitions (16°C, 10L:14D) inhibited the expression of autogeny among the female progeny of field-collected *Culex tarsalis* females. The same F_1 progeny expressed 43% autogeny when reared in an insectary under simulated "summer" conditions (25°C, 16L:8D). Diapausing F_1 progeny did not express autogeny after a 42-day simulated winter or after diapause termination periods of 4 days in the insectary or 29 days outdoors during January. Females were then blood fed and the progeny reared in the insectary; autogeny rates among F_2 females in 4 families ranged from 0 to 43%. Autogeny rates among females emerging from pupae collected during March at 2 localities in Kern County, California were 15 and 17%.

Diapause was not induced uniformly among females from a laboratory-selected autogenous colony. Autogeny rates before and after a simulated winter period did not change significantly.

INTRODUCTION

The expression of autogeny in *Culex tarsalis* Coquillett females culminates the interaction of genetic and environmental factors which may vary over space and time. In California, autogeny rates vary geographically among field populations (Hardy and Reeves 1973, Moore 1966[2]) and appear to be governed by one or more dominant, autosomal genes (Moore 1966,[2] Eberle and Reisen 1986). Autogeny rates also vary seasonally and approach 0% during fall when field populations enter hibernation (Moore 1963, Spadoni et al. 1975). Laboratory experiments have verified that when genetically autogenous immatures are exposed to cool temperature (Reisen et al. 1984) and/or short day length (Harwood 1966, Reisen et al. (1986a), the frequency of autogeny among the emerging females decreases. Unfortunately, these studies were not designed to determine if the frequency of the genes coding for autogeny changed or if changing environmental factors altered the penetrance of the genes for autogency.

The present study presents a probable mechanism by which genetically autogenous females overwinter in California.

METHODS AND MATERIALS

STRAINS. Two strains of *Cx. tarsalis* originating from Breckenridge, Kern County, California were used for experimentation.

$BrWF_1$: The F_1 progeny from females collected at CO_2-baited traps during October 1984.

BrAut: A laboratory colony selected for autogeny from founders collected at Breckenridge during October 1980 (Eberle and Reisen 1986).

DIAPAUSE INDUCTION. Five pans (23 × 36 cm) of each strain were reared in an insectary (16L:8D, 25°C) at a density of 200 first instar larvae (L1)/pan and fed a diet of ca. 0.5 g of powdered rat chow per pan per 2 days until pupation. At first pupation, the pupae from 4 pans per strain were discarded and the remaining larvae transferred to a bioenvironmental chamber set at 10L:14D and 16°C. Pupae in the remaining pan per strain were allowed to emerge in the insectary as a nondiapause control to estimate autogeny rates under "summer" conditions. Adults were held under at the same conditions under which they pupated and were offered 10% sucrose solution on cotton pledgets. Approximately 1 and 2 weeks after pupation in the insectary and bioenvironmental chamber, respectively, a sample of females was dissected to determine follicular maturation. The lengths of 5 1° and 2° follicles per female with 1° follicles at ≤ Stage I-II were measured at 400X. Females with follicles matured to Stage V were considered autogenous and were not measured.

Since the $BrWF_1$ mosquitoes were not selected for mating under laboratory conditions, 75 $BrWF_1$ females were force-mated to individual $BrWF_1$ males using the methods described by Baker (1964). The remaining $BrWF_1$ mosquitoes were allowed to cohabit 3.8 liter (1

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[2] Moore, C. G. 1966. Environmental factors influencing the proportion of autogenous ovarian development in populations of the mosquito *Culex tarsalis* Coq. PhD dissertation, University of California, Davis, Calif. 105 pp.

gal) cages and mate *en masse*. All mosquitoes were held for a simulated winter period (10 L:14D, 16°C) which was terminated after 42 days.

DIAPAUSE TERMINATION. At the end of the simulated winter period, a sample of females from both strains was dissected to determine reproductive status. The remaining adults were transferred to the insectary (16L:8D, 25°C) where they were held 4 days for diapause termination. One group of $BrWF_1$ females was held outdoors from December 27, 1984 to January 25, 1985, where they repetitively were offered a restrained chick as a blood meal source. After the diapause termination period, surviving $BrWF_1$ females from the *en masse* mating group were dissected to determine if diapause termination conditions initiated autogenous egg development. Follicles were measured to ascertain if diapause had been terminated. The forcemated $BrWF_1$ females were offered a restrained chick until they either took a blood meal or died. Blooded females were held until gravid and then placed in vials with tap water for oviposition. Egg rafts were reared as single families. Females from each family were maintained on 10% sucrose in the insectary for more than 5 days and then dissected to determine follicular maturation.

FIELD OBSERVATIONS. The F_1 progeny of overwintering females were collected as pupae from seepage pools along the Kern River and from flooded hoofprints and puddles at Poso West near Bakersfield, Kern County, California on March 19 and 26, 1985, respectively. Pupae were allowed to emerge under natural temperature and photoperiod and the resulting adults were maintained on 10% sucrose solution in the insectary. After 5–7 days, females were dissected to determine follicular maturation. Water temperature within 0.5 cm of the surface was recorded at 30 min. intervals in a 16 cm deep, sunlit pool at Poso West from February 19 to March 20, 1985.

RESULTS

DIAPAUSE INDUCTION. The autogeny rate among $BrWF_1$ females emerging in the insectary was 43%, while the autogeny rate among females emerging in the bioenvironmental chamber was 0% (Table 1). Follicular maturation of females emerging in the bioenvironmental chamber was arrested uniformly at Stage I. Females in the insectary were not in diapause, since vitellogenesis had progressed to or beyond Stage I–II, and the length of the 1° follicle was significantly longer and the 1°/2° follicular length ratio was significantly greater than females emerging in the bioenvironmental chamber (Table 1).

The autogeny rate of the BrAut females decreased from 95% in the insectary to 42% in the bioenvironmental chamber (Table 1). Follicular maturation among females in the bioenvironmental chamber was not arrested uniformly: among a sample of 25 females, 11, 2, 4 and 8 had 1° follicles matured to Stages V, IIa, I-II, and I, respectively. In addition, the 1° follicle length and the 1°/2° follicular length ratio of the 8 females at Stage I was significantly larger than similar measurements of $BrWF_1$ females. Six of 8 BrAut females had follicular ratios of <1.5 and were considered to have entered diapause.

DIAPAUSE TERMINATION. Female reproductive status did not change during the 42-day simulated winter period. Only 1 of 20 $BrWF_1$ females from the *en masse* mated group had initiated vitellogenesis beyond Stage I. The mean length of the 1° follicle and the mean 1°/2° follicular length ratio were not significantly different from females dissected at diapause in-

Table 1. Effects of diapause induction and termination cues on ovarian development in field and laboratory strains of *Culex tarsalis*.

Treatment	Strain	Stage 1 or I–II			Autogeny	
		1° foll[1]	10/20 ratio	n	%[2]	n
Insectary, emergence	$BrWF_1$	79.3	1.81	15	43	60
(16L:8D, 25°C)	BrAut	—	—	—	95	41
Chamber[3], emergence	$BrWF_1$	39.4	1.06	15	0	45
(10L:14D, 16°C)	BrAut	57.6	1.38	8	42	60
42 days in chamber	$BrWF_1$	39.9	1.18	20	0	20
	BrAut	58.5	1.50	10	48	25
4 days in insectary	$BrWF_1$	44.4	1.33	13	0	13
29 days outdoors	$BrWF_1$	51.5	1.53	5	0	5

[1] 1° foll = mean length of the primary follicle in μ for n females which did not mature their ovaries autogenously. 1°/2° ratio = primary/secondary follicular length ratio. —=none measured.

[2] Percentage of the total females dissected (n) that matured their eggs autogenously. n = number of females examined.

[3] Chamber = bioenvironmental chamber.

duction (Table 1). Survivorship of the $BrWF_1$ adults during the 42-day simulated winter period was greater for females (58%, n = 182) than males (2%, n = 124), while forced-mated females survived best (71%, n = 75).

Similarly, the reproductive status of 25 BrAut females dissected at diapause termination was not markedly different from females dissected at diapause induction, where 12, 2, 10 and 1 females were at follicular Stages V, I–II, I and N, respectively. The mean length of the 1° follicle and the mean 1°/2° ratio of females dissected at diapause termination were not larger than for females dissected at diapause induction (Table 1). Survivorship of the BrAut adults (females = 31%, n = 105 and males = 1%, n = 141) was poorer than that of the $BrWF_1$ adults.

Experimental diapause termination was hampered by excessive mortality. Only 13 of 30 (15%) $BrWF_1$ females in the *en masse* mated group which were transferred to the insectary for diapause termination survived for 4 days and were dissected; all were still at Stage I, although the length of the 1° follicle and the follicular length ratio were significantly larger than those of females dissected at diapause termination (Table 1). Similarly, only 5 of 55 (9%) $BrWF_1$ females which were held outdoors survived 29 days from December 27, 1984 to January 25, 1985 and were dissected; all were still at Stage I, but the length of the 1° follicle and follicular length ratio were significantly larger than females which were held for 4 days in the insectary (Table 1). Females held outdoors were offered a restrained chick suspended from the cage lid on 5 nights from January 10 to 25, but none took a blood meal. Mean air temperature for Bakersfield during January was 6.1°C (range = 2.2 – 10.6°C), while daylength increased from 9.8 to 10.3 hours.

Only 25 force-mated $BrWF_1$ females survived the 4-day diapause termination period and were offered a restrained chick. Of these 11 blood fed over the subsequent 10 day period, but only 4 successfully oviposited. All females that emerged from each family and survived for a 5-day period on 10% sucrose were dissected to determine autogeny status. Autogeny rates in each family were 43% (n = 7 females dissected), 38% (n = 74), 15% (n = 96) and 0% (n = 1). Variability in the numbers dissected was attributed to the small size of the egg rafts and the low percentage hatch related to forcemating. Thus, 3 of 4 females which failed to develop their eggs autogenuously during "winter" or during conditions terminating diapause, imbibed a blood meal and produced some progeny which matured their follicles autogenously.

FIELD OBSERVATIONS. Overall, 15% (n = 54) and 17% (n = 65) of females collected as pupae from Kern River and Poso West, respectively, exhibited follicular maturation to Stage V and were considered autogenous. These females emerged from the first *Cx. tarsalis* pupae collected in the Bakersfield area in late winter and were considered to be the F_1 progeny of overwintering females that terminated diapause and oviposited during February 1985 (unpublished data). The overall mean water temperature recorded at Poso West was 12.6°C (daily range = 8.1 – 17.7°C). Daylength increased from 10.5 hr on February 1 to 12.4 hr on March 26, 1985.

DISCUSSION

The present observations describe a possible mechanism by which autogenous *Cx. tarsalis* females may overwinter. Cool temperature (16°C) and short daylength (10 hr) inhibited the penetrance of autogeny and arrested follicular maturation at Stage I. Females remained at Stage I throughout winter and into the diapause termination period. Although mean 1° follicular length and the follicular length ratio did increase, vitellogenesis did not progress beyond Stage I-II or II, when $BrWF_1$ females were either exposed to warm temperature (25°C) and long daylength (16 hr) in the insectary or gradually lengthening days (9.8 to 10.3 hr) and outdoor temperatures (mean = 6.1°C). Once inhibited environmentally, genes coding for autogeny do not express themselves, even under diapause termination conditions which enable follicular maturation to progress to Stage I-II prior to host-seeking and to Stage V after a replete blood meal is imbibed. Thus, genetically autogenous females become functionally anautogenous under autumnal conditions and remain so even under diapause terminating conditions. However, the progeny of these functionally anautogenous females readily expressed autogeny when reared under long daylength and warm temperature in the insectary. Interestingly, females emerging from wild-caught pupae which were reared under periods of short, but lengthening, photoperiods and cool temperature during late winter (February/March) expressed autogeny upon emergence. These conditions were similar to the photoperiods and temperatures experienced by larvae pupating during November when autogeny was arrested as emerging females entered diapause (Reisen et al. 1986a). A critical difference appears to be the exposure to lengthening photoperiods in the spring as opposed to shortening photoperiods during autumn.

Research on hibernation in *Cx. tarsalis* has been complicated by the failure of laboratory-selected strains to uniformly enter diapause (Reisen et al. 1986a). In the present study, approximately half of the BrAut females, which originated from the same locality as the $BrWF_1$ females, failed to uniformly respond to diapause induction cues and exhibited 40–50% autogeny rates. Thus, genomes failing to enter diapause must persist in nature and are selected during colonization. The existence of these genotypes in California was suggested by the variability in ovarian morphometry exhibited by females collected during winter in Kern County (Reisen et al. 1986b).

Most research on the physiological ecology of overwintering among *Culex* species capable of autogeny has been restricted to the *Cx. pipiens* complex. Within this complex the expression of autogeny and the ability to enter diapause is variable among taxa. Anautogenous *Cx. pipiens* Linn. readily enter diapause under appropriate conditions of shortening daylength and cool temperature (Eldridge 1968, Spielman and Wong 1973, Vinogradova 1960). However, autogenous *Culex pipiens* strains in both Europe and North America do not arrest vitellogenesis and become gravid regardless of daylength or temperature conditions (Vinogradova 1960, Spielman and Wong 1973). In addition, the southern taxon, *Cx. quinquefasciatus* Say, appears to be unable to enter diapause, but rather may enter quiescence (Teckle 1960, Eldridge 1968). *Culex tarsalis* appears to have evolved different overwintering mechanisms whereby autogeny is expressed facultatively and inhibited by diapause induction cues. Both autogenous egg development and the ability to undergo diapause appear to be genetically variable traits in Kern County.

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