STUDIES ON AUTOGENY IN *CULEX TARSALIS*: 3. LIFE TABLE ATTRIBUTES OF AUTOGENOUS AND ANAUTOGENOUS STRAINS UNDER LABORATORY CONDITIONS[1]

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ABSTRACT. The reproductive biology and life table attributes of autogenous and anautogenous strains of *Cx. tarsalis* which were selected from the same parent colony were compared under laboratory conditions. Autogenous mosquitoes required 1 day longer to complete immature development, but oviposited 1 to 2 days earlier than anautogenous mosquitoes. Autogenous females readily imbibed blood meals from restrained chickens if ovarian maturation had not progressed to Christophers' Stage III. Wing length and life expectancy were not significantly different between strains; however, autogenous females laid a significantly smaller number of eggs per raft during initial oviposition than anautogenous females. Egg raft size did not differ significantly between strains during subsequent ovipositions resulting in similar net reproductive rates (R_o). Earlier oviposition and a comparable R_o resulted in a greater intrinsic rate of increase (r_m) and birth rate (b) for autogenous than anautogenous cohorts. Thus, highly autogenous populations would be able to exploit newly created surface water breeding sources more rapidly than highly anautogenous populations. However, highly autogenous populations probably would not be able to transmit a horizontally maintained arbovirus as efficiently as anautogenous populations, since autogenous females imbibe their initial blood meal later in life than anautogenous females.

INTRODUCTION

Some *Culex tarsalis* Coquillett females oviposit their initial batch of eggs without imbibing a blood meal, i.e., autogenously (Bellamy and Kardos 1958), although blood meals are required for subsequent ovipositions (Nelson and Milby 1982). Autogeny appears to be inherited as a dominant genotype (Eberle and Reisen 1986) which is expressed facultatively depending upon environmental conditions (Reisen et al. 1984, Reisen 1986). Field and laboratory observations indicate that autogenous populations may oviposit earlier in life and thus have a faster intrinsic rate of increase (r_m) than populations which produce their first egg batch anautogenously. In agreement, large *Cx. tarsalis* populations frequently associated with seasonal flooding usually have high rates of autogeny (e.g., Lyness 1970). These apparent changes in life table attributes associated with autogeny are difficult to study quantitatively under field conditions and previously have not been well studied under laboratory conditions.

The present research describes the life table attributes of autogenous and anautogenous strains of *Cx. tarsalis* under laboratory conditions. Between strain variation in life table attributes were circumvented, since both strains originated from the same parent colony.

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MATERIALS AND METHODS

Strains. The autogenous Br83 (Br83Aut) and anautogenous Br84 (Br84An) strains were selected from the Br80 laboratory colony which was established from females collected at Breckenridge Road, Kern County, California during October 1980 (Eberle and Reisen 1986). More than 90% of Br83Aut females exhibit autogeny when reared at uncrowded densities of <300 1st instar larvae (L1) per pan. Br84An females exhibit <10% autogeny regardless of rearing conditions.

Life table methods. Four replicates of 200 L1 <24 hours old were established per group in 23 × 36 cm enameled pans from >100 egg rafts hatched *en masse*. Larvae were reared and adults maintained in an insectary at 25 ± 2°C and 16L:8D photoperiod with 1 hr simulated crepuscular periods. Water temperature in the rearing pans ranged from 20 to 24°C. Larvae were fed 0.5 gm of powdered rodent chow on alternate days until first pupation. Pupae were counted daily and transferred to emergence cups from which the emerging adults were counted daily and removed.

Three cohorts of 25 males and 25 females which emerged on the same day were established in paper cages (lid diameter = 21 cm, bottom diameter = 16 cm, height = 21 cm) for each of three groups. Each cohort continually was offered 10% sucrose solution on a cotton pledget as a carbohydrate source and a cup filled with tap water as an oviposition substrate. Initially cohorts of Br83Aut (Br83Aut-1) and Br84An mosquitoes were offered a restrained chick daily as a blood meal source from emergence until death. Unexpectedly, many of the genetically

autogenous Br83Aut females imbibed a blood meal confounding our evaluation of the impact of autogenous oviposition on life table attributes. Subsequently, a second group of Br83Aut adults (Br83Aut-2) were evaluated as above except that cohorts were not offered a blood meal until after >80% of the females had oviposited their initial egg batch autogenously. Cages were examined daily until all the adults had died. Dead adults were removed, counted and their wing lengths measured. Egg rafts were removed, counted and placed individually into 6 dram vials with tap water for hatching. After three days rafts were counted to determine the numbers of total and hatched eggs per raft. Females from each strain which were not used in the life table experiments were maintained on 10% sucrose for >5 days and then dissected to determine autogeny status. Females with primary follicles matured to Christophers' (1911) Stage V were considered to be autogenous.

Rate of ovarian development. Ten females each from the Br83Aut and Br84An strains were dissected each morning on days 1 to 6 of adult life and the degree of follicular maturation classified using the scheme of Christophers' (1911) as modified by Mer (1936).

Age at blood feeding. Four cohorts each of 25 Br83Aut and 25 Br84An females were offered a restrained chick suspended from the lid of each cage daily from 12 to 36 hr postemergence until >90% of the females blood fed or were 6 days old. Blood fed females were removed daily and counted. A sample of blood fed females from each strain was dissected at 24–48 and 96–144 hr postfeeding to determine the degree of follicular maturation.

Statistics. Median emergence times (E_{50}) were estimated from the cumulative emergence curves by probit analyses. The formulae and computer programs described previously (Reisen et al. 1979, 1984) were used to calculate life table parameters including life expectancy at emergence (e_x in days), net reproductive rate (R_o in females per female per generation), the mean age of reproduction (T_o in days), the intrinsic rate of increase (r_m in females per female), the instantaneous birth rate (b in females per female) and generation time (G in days). R_o, r_m and B were calculated using adult (series A) and adult plus immature (series B) attributes. For series A, all eggs became adults instantaneously; i.e., immature survival was 100% and the time for oviposition to emergence required 0 days. For series B, estimates of immature survival and developmental rate were derived from rearing experiments. Comparisons among means were made by analysis of variance procedures (Sokal and Rohlf 1969).

RESULTS

Immature development. Immature survivorship from L1 to adult emergence was higher for the Br83Aut strain than for the Br84An strain during Exp. 1, but not Exp. 2 (Table 1). Br83Aut development appeared to be about 1 day slower than Br84An development based on median emergence times for both males and females in paired rearing experiments (Exp. 1 and 2, Table 1). Differences in developmental times among experiments were attributed to slight and uncontrollable differences in room temperature.

Rate of follicular maturation. Follicular maturation progressed more rapidly for Br83Aut than Br84An females (Table 2). When 24–48 hr old, 50% of the Br83Aut strain females were at Stage II, while vitellogenesis was not yet detected in the Br84An females. Maturation to Stage V was completed 96–144 hr after emergence at insectary temperatures which ranged from 23 to 25°C. By 48–72 hr postemergence, 90% of Br84An females had initiated vitellogenesis to ≥ Stage I–II. Follicular maturation of

Table 1. Comparative survivorship, developmental time and percent autogeny of autogenous (Br83Aut) and anautogenous (Br84An) strains of *Culex tarsalis*

			Developmental time (days)[c]						
			Males			Females			
Exp[a]	Strain	%Surv[b] (L1-A)	E50	E90	90/50	E50	E90	90/50	Autog. %(n)
1	Br83Aut	55.5a	18.6a	20.4	1.10	19.8a	21.8	1.10	100 (93)
	Br84An	38.4b	17.7a	20.0	1.13	18.7a	20.4	1.09	11 (35)
2	Br83Aut	63.0a	16.2a	17.6	1.09	17.3a	19.0	1.10	99 (75)
	Br84An	62.4a	15.5b	16.6	1.07	16.5b	17.9	1.08	4 (45)
3	Br83Aut	53.3	15.0	16.3	1.08	16.0	17.7	1.11	100 (75)

[a] N = 4 pans per strain for Exp. 1 and 2 and 3 pans per strain for Exp. 3.

[b] Percent survivorship from 1st instar (L1) through adult emergence (A). Means in the same column in the same experiment followed by the same letter were not significantly different ($P > 0.05$) when tested by an ANOVA with pans blocked by shelf position.

[b] Mean time in days from eclosion to 50 and 90% male and female emergence estimated from the cumulative emergence curves for each pan by probit analyses and compared by ANOVA.

Table 2. Rate of follicular maturation in autogenous and anautogenous *Culex tarsalis* females under insectary conditions (23–25°C, 16L:8D).

Age (hr)	Number of females in Christophers' stages							
	N	I	I-II	IIA	IIB	III	IV	V
UNFED								
Br83 autogenous strain								
0–24	7	3						
24–48		1	4	5				
48–72					3	7		
72–96						10		
96–120						1	8	1
120–144							2	7
Br84 anautogenous								
0–24	5	5						
24–48		10						
48–72		1	6	2	1			
72–96			5	3				
BLOOD FED								
Br83 autogenous								
24–48			1	3	13	1		
96–144						2	1	10
Br84 anautogenous								
24–48		4	6	3	3			
96–120						2	9	4
120–144							1	8

Br84An females was arrested at Stage I–II or II until the females imbibed a blood meal.

Age at blood feeding. Females were first offered a blood meal 12–36 hr postemergence. By the following morning 50% (n = 98) of Br83Aut and 78.2% (n = 69) of Br84An females had blood fed (Fig. 1). When dissected, 78% Br83Aut females (n = 18) had matured their follicles to ≥Stage IIb, while only 19% (n = 16) Br84An females had matured their ovaries to Stage IIb (Table 2). On the following night, 98% (n = 40) of the remaining unfed Br84An females blood fed, while only 8% (n = 47) Br83Aut females blood fed (Fig. 1). Thus, it appeared that although young autogenous females may blood feed, relatively few females will imbibe a blood meal after vitellogenesis progresses autogenously to ≥Stage III.

Life table attributes. The wing lengths of adults used in the life table cohorts did not differ significantly among groups (Table 3), even though significant differences in rearing success and developmental rates were observed (Exp. 1 and 3, Table 1). The autogeny rate of Br83Aut females was 100% and significantly greater ($P < 0.01$) than the 11% estimated for the Br84An females (Table 3).

Life expectancy at emergence, e_x, for both males and females did not differ significantly among groups (Table 3). Survivorship (l_x) remained greater than 0.9 for 16, 8 and 14 days postemergence for Br83Aut-1, Br83Aut-2 and Br84An females, respectively. Female survivorship for all groups approximated a Type I curve of Slobodkin (1962) with mortality concentrated late in life.

The mean number of eggs/raft laid by Br83Aut-1 and -2 females was significantly less than laid by Br84An females (Table 3). Ranges in egg raft size overlapped (Br83Aut-1 = 11 to 432, Br83Aut-2 = 18 to 438, and Br84An = 27 to 481 eggs/raft). The mean number of eggs laid per raft by Br83Aut females during the initial oviposition on days 4–6 (Br83Aut-1 = 174.0, n = 58, and Br83Aut-2 = 157.7 eggs/raft, n = 58) was significantly less ($P < 0.01$) than laid by Br84An females during days 5-7 (237.1 eggs/raft, n = 51). However, the number of eggs per raft during the second oviposition did not differ significantly among groups when all females blood fed (days 9–11, Br83Aut-1 = 264.6, n = 51; Br83Aut-2 = 262.8, n = 65; Br84An = 267.6 eggs/raft, n = 34). Mean egg raft size for Br83Aut-1 and -2, but not Br84An, was significantly ($P < 0.01$) greater during oviposition 2 than oviposition 1. Unexpectedly, the ingestion of blood meals by >50% of the Br83Aut females in Br83Aut-1 did not markedly increase mean egg raft size as compared to Br83Aut-2 during initial oviposition. Egg raft size decreased significantly ($P < 0.01$) as a function of age for the Br84An females (r = −0.30), but not for the Br83Aut-1 and -2 (r = −0.06 and −0.07, respectively). The number of rafts oviposited per cohort was higher for the Br83Aut than for the

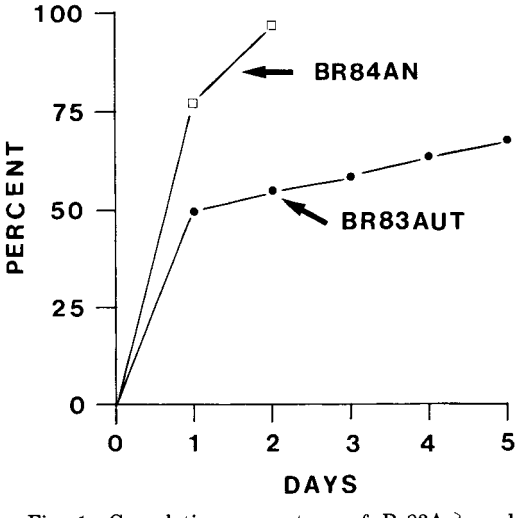

Fig. 1. Cumulative percentage of Br83Aut and Br84An females blood feeding on a restrained chick suspended from the lid of a bucket cage (total of 4 groups of 25 females/cage/strain).

Table 3. Mean life table attributes of autogenous strain females offered (Br83Aut-1) or not offered (Br83Aut-2) a blood meal for initial oviposition and anautogenous strain females (Br84An) of *Cx. tarsalis* (n = 3 cohorts of 25 males and 25 females per strain).

Attribute	Groups			
	Br83Aut-1	Br83Aut-2	Br84An	ANOVA[a]
Adult status				
Male wing length in mm (n)	3.45 (62)	3.55 (32)	3.47 (71)	ns
Female wing length in mm (n)	4.10 (66)	4.16 (65)	4.13 (72)	ns
Autogeny rate in % (n)	100 (93)a	100 (75)a	11 (35)b	
Survivorship				
Male life expectancy (days)	22.3	20.6	24.4	ns
Female life expectancy (days)	22.2	22.5	22.6	ns
Reproductive characteristics				
Number of rafts/cohort	91.7	96.3	77.3	ns
Eggs/raft (n)	207.4 (275)b	217.7 (289)b	232.5 (232)a	**
Percent hatch	61.6	70.2	63.5	ns
Life table characteristics				
T_o (days)	13.3	13.8	14.2	ns
G (days)	36.9a	32.7b	37.4a	*
Series a				
R_o (females/female/generation)	396.9	473.7	428.7	ns
r_m (females/female)	0.805b	0.903a	0.753b	*
b (females/female)	1.33ab	1.53a	1.15b	*
Series b				
R_o (females/female/generation)	220.3a	249.4a	164.6b	*
r_m (females/female)	0.145b	0.179a	0.136c	*
b (females/female)	2.23a	2.30a	1.89b	*

[a] Group means were tested by analyses of variance (ANOVA) with * $0.01 < P < 0.05$, ** $P < 0.01$, ns $P > 0.05$. Means in the same row followed by the same letter were not significantly different using a multiple range test ($P > 0.05$).

Br84An strain females, although the means were not significantly different due to within strain variability in egg raft production. Fertility (% hatch) was comparable for all groups (Table 3), and decreased significantly ($P < 0.01$) as a function of female age at oviposition (Br83Aut-1, r = −0.24, Br83Aut-2, r = −0.28 and Br84An r = −0.27).

Although the rate of follicular maturation was faster (Table 2) and the time of initial oviposition occurred about 1–2 days earlier (Fig. 2) for the Br83Aut-1 and -2, the mean age of cohort reproduction (T_o) did not differ significantly among groups (Table 3). Faster immature development, E_{50} (Exps. 1 and 3, Table 1), combined with comparable estimates of T_o resulted in faster estimates of mean generation time, G, for Br83Aut-2 than Br83Aut-1 and Br84An (Table 3). For series B calculations, a (L1 to A survivorship) was 0.555, 0.533 and 0.384 and d (rough estimate of age from egg hatch to 1st oviposition) was 26.8, 22.0 and 25.5 days for Br83Aut-1, Br83Aut-2 and Br84An, respectively. The sex ratio for all rearings, 1.045:1 males:female (n = 2593), did not differ significantly from 1:1 ($\chi^2 = 1.096$, $P > 0.05$). Thus, estimates of the daily reproductive rate in females per female per day (m_x) were based on total eggs oviposited × 0.5. The addition of immature survivorship (series B) markedly reduced the estimates of the net reproductive rate, R_o (Table 3). Group estimates were not significantly different using series A, but Br84An was significantly less than Br83Aut-1 and -2 using series B calculation procedures. Earlier oviposition combined with a comparable or greater R_o resulted in a greater innate rate of increase, r_m, for the Br83Aut-1 and -2 than for the Br84An strain using both series A and B calculation methods (Table 3). Increased r_m also was reflected in the higher birth rate, b, estimated from the stable age distribution.

DISCUSSION

Autogenous females required about a day longer than anautogenous females to develop to adults, presumably because of the greater nutritional requirements of the females to develop the initial egg batch without a blood meal. Surprisingly, similar slower developmental rates were exhibited by the Br83Aut males which presumably did not have the increased nutritional requirements related to egg production. Since both strains were selected from the same parent colony, background genotypes were presumed to be similar and the observed differences related to the genes coding for autogeny. Genetically autogenous males in the *Culex pipiens* complex contained increased nutritional re-

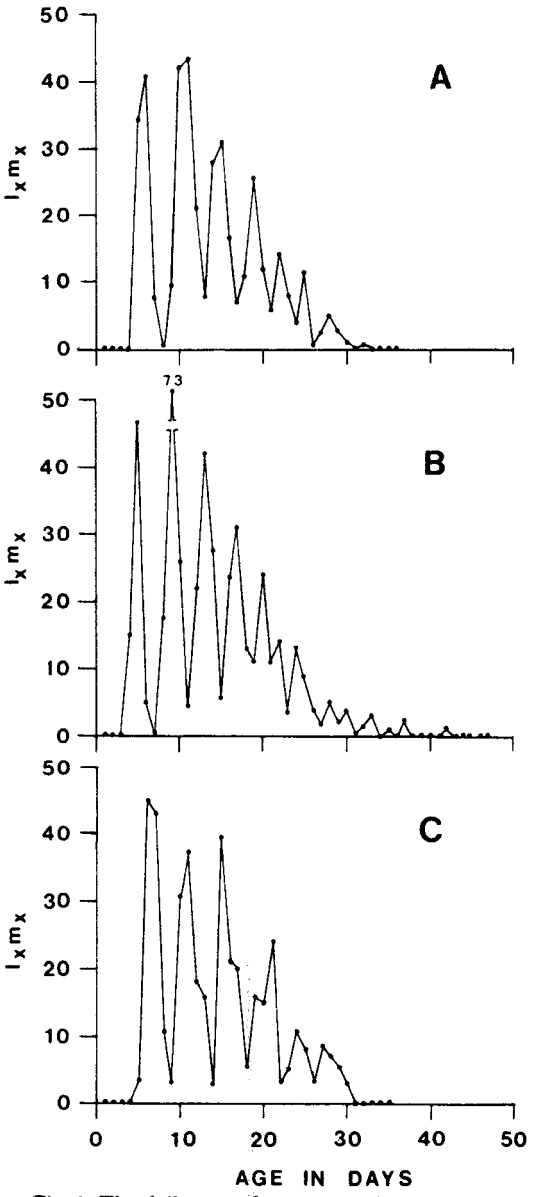

Fig. 2. The daily reproductive rate ($l_x m_x$) in female eggs per female per day of (A) Br83Aut-1, (B) Br83Aut-2 and (C) Br84An *Culex tarsalis* females in paper cages (3 cohorts of 25 males and 25 females per strain) plotted as a function of cohort age in days after emergence.

serves, leading Rozeboom and Twohy (1958) to conclude that the accumulation of nutritional reserves during 4th instar is a physiological function shared by both males and females and is independent of egg maturation. However, in their study the autogenous males also were larger than the anautogenous males which was not the case in the present study. Further research is necessary to characterize the energy reserves of genetically autogenous *Cx. tarsalis* male mosquitoes.

Autogenous females oviposited 1–2 days sooner than anautogenous females under the present laboratory conditions. We felt that this time period represented a minimal physiological difference and that the actual time of oviposition by anautogenous females in nature would be further delayed due to the time required for host-seeking. Previous mark-release-recapture experiments supported this premise and suggested that anautogenous females did not blood feed until later in life delaying initial oviposition by 2–3 days (Nelson and Milby 1982, Reisen et al. 1983). When dissected, most females collected in nature at CO_2-baited traps had matured their follicles to Stage I–II or IIa, the host-seeking resting stage, and were inseminated (Reisen et al. 1983); while in the laboratory the Br84An females blood fed at Stage I when they were 24–48 hr old.

The mean number of eggs oviposited per raft initially by the Br83Aut-1 females was not significantly greater than the number per raft oviposited by Br83Aut-2 females, even though 50–60% of the Br83Aut-1 females had imbibed a blood meal. The number of follicles destined to mature seemed to be determined autogenously at emergence, since mosaic maturation or large scale follicular degeneration was not observed among Br83Aut females dissected 96–144 hr after blood feeding (Table 2). Blood feeding by autogenous *Cx. tarsalis* females in the laboratory was reported previously by Bellamy and Corbet (1974) and appears to be a laboratory phenomenon associated with bringing the blood meal host into close proximity with the resting females. Spadoni et al. (1974) found that practically all host-seeking females in a highly autogenous field population were anautogenous or parous; i.e., few females collected in CO_2 baited traps developed their eggs autogenously after being held for 5–10 days on 10% sucrose. Once the ovary developed autogenously to >Stage III, few Br83Aut females blood fed even under the present laboratory conditions. A similar situation must persist in nature for few blood engorged females collected resting in nature have been found to contain mature Stage V ovaries (Nelson and Milby 1982, Reisen et al. 1983).

Earlier oviposition and an equal (series A) or greater (series B) net reproductive rate resulted in a greater innate rate of increase for the Br83Aut strain than for the Br84An strain. Interstrain differences in values of R_o and r_m would be enhanced in nature where daily survivorship is considerably less than observed under laboratory conditions. In the present study, daily survivorship was high (Br83Aut-1 = 0.958, Br83Aut-2 = 0.978, and Br84An = 0.961) and

approximate the Type I survivorship curve of Slobodkin (1962) with mortality concentrated late in life after the reproductive period. Conversely, daily survivorship estimated in nature ranged from 0.65 to 0.85 using vertical age-grading or horizontal release-recapture estimation methods (Nelson et al. 1978, Milby et al. 1983, Reisen et al. 1983) and approximated the Type III survivorship curve with the mortality rate constant and independent of age. Earlier autogenous oviposition would be advantageous for field populations exhibiting a Type III curve, since more females would survive to oviposit. For example, a field population with a constant daily survivorship of 0.8 would lose about 46% of the total females over a 2 day period, the estimated delay in initial oviposition by an anautogenous population. Enhanced reproduction earlier in adult life would result in a greater intrinsic rate of increase which would enhance the ability of autogenous populations to exploit newly formed breeding sources.

Although autogenous populations may rapidly attain elevated abundance levels, highly autogenous populations may vector a horizontally transmitted arbovirus less efficiently than anautogenous populations. The age at which the initial blood meal is imbibed is delayed several days in autogenous populations until after the initial egg raft is oviposited (Reisen et al. 1983). Thus, large *Cx. tarsalis* populations that frequently have been associated with widespread seasonal flooding may not be accompanied consistently by increased arbovirus transmission. In agreement, the flooding of Kern County by the Kern River during 1962 (Reeves et al. 1964), 1969 (Lyness 1970) and 1983 (Reisen 1984) was associated with increases in *Cx. tarsalis* abundance without encephalitis attack rates comparable to the epidemic of 1952 (Reeves and Hammon 1982) or the impending epidemic of 1958 (Reeves et al. 1964). Increased *Cx. tarsalis* abundance during 1969 and 1983 was associated with elevated autogeny rates. The depressive effect of elevated autogeny rates on encephalitis virus transmission rates also was projected from the statistical simulation studies of Moon (1973).[2]

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