STUDIES ON AUTOGENY IN *CULEX TARSALIS*: 1. SELECTION AND GENETIC EXPERIMENTS[1]

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ABSTRACT. Autogenous and anautogenous strains were selected from a laboratory colony of *Culex tarsalis* established from a foothill environment in Kern County, California. An autogenous strain also was selected from immatures collected at the Kern River. Autogenous and anautogenous strains remained heterogeneous and did not consistently exhibit 100 or 0% autogeny, respectively, despite continual selection pressure and inbreeding.

Autogeny rates did not increase when sublines selected from the anautogenous strain were outcrossed within single female lines; however, autogeny rates increased when crosses were made between single female lines. Crosses and backcrosses between the autogenous and anautogenous strains indicated that autogeny was controlled by a dominant, autosomal gene(s). The persistence of heterogeneity during selection and the decrease in autogeny rates among the female progeny of crosses where autogeny was inherited through the male indicated that the genetics of autogeny may be polyfactorial and/or phenotypic expression compromised by sex.

INTRODUCTION

Some *Culex* mosquitoes, including *Culex tarsalis* Coquillett, may mature an initial egg batch autogenously (i.e., without imbibing a blood meal), although blood meals are required for subsequent ovipositions (Nelson and Milby 1982). Autogeny is important epidemiologically, since the initial bloodmeal is delayed, thereby reducing the number of potentially infective females (Reisen et al. 1983). Conversely, autogenous females oviposit earlier in life increasing the growth rate and size of the population.

The penetrance and expression of autogeny in *Cx. tarsalis* have been related experimentally to temperature, photoperiod, nutrition and larval crowding (Kardos 1959, Chaniotis 1960[3], Harwood 1966, Moore 1966[2], Spadoni 1974[4], Reisen et al. 1984). In nature the prevalence of autogeny varies spatially (Hardy and Reeves 1973) and temporally (Moore 1963, Spadoni et al. 1974, Reisen et al. 1983). In addition, colonies of *Cx. tarsalis* typically retain their autogenous character (Chao 1958, Bellamy and Kardos 1958, Moore 1966[2]) suggesting there is a genetic basis for the variability observed in nature. Moore (1966[2]) crossed strains exhibiting high and low rates of autogeny and found that the F1 hybrids exhibited high autogeny rates. He postulated that autogeny in *Cx. tarsalis* may be a dominant character, but cautioned against this interpretation, since his crosses were made with heterogeneous strains.

The present study describes the selection for autogenous and anautogenous strains from a single laboratory colony, and the results of preliminary crosses to elucidate the mode of inheritance. Emphasis was placed on discerning a genetic basis for autogeny under controlled conditions which minimized environmental factors that could alter penetrance.

MATERIALS AND METHODS

Strains. Autogenous (aut) and anautogenous (an) strains were selected from 2 laboratory colonies. Both strains were colonized and maintained in 45 × 45 × 45 cm cages in an insectary (25±2°C, 16L:8D) and initially were offered a restrained chick at weekly intervals as a blood meal source.

The Br80 colony was established during the fall of 1980 from females collected by CO_2 baited traps at Breckenridge, 20 km E of Bakersfield in the arid Sierra Nevada foothills. The autogeny rate at Breckenridge averaged 28% (range = 6–49%) during the summer of 1981 (Reisen et al. 1983).

The KR83 colony was established in May 1983 from larvae and pupae collected from overflow pools along the Kern River 10 km W of Bakersfield. The autogeny rate among fe-

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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, Univ. Calif., Davis, 105 pp.

[3] Chaniotis, B. N. 1960. Autogeny in a colony of *Culex tarsalis* Coq. as affected by the level of protein in the larval diet. MS Thesis, Univ. Calif., Davis, 41 pp.

[4] Spadoni, R. D. 1974. Aspects of autogeny in *Culex tarsalis* Coquillett in Butte and Glenn Counties, California, MA Thesis, San Jose State University, San Jose, 153 pp.

males emerging from pupae collected in May was 84% (n = 50) (Reisen 1984).

Autogeny rates were determined by dissection after offering cohorts of newly emerged females 10% sucrose for more than 5 days. Females having ovaries with more than one follicle matured to ≥ Stage IV of Christophers (1911) were considered to be autogenous, while females with all ovarioles matured to ≤ Stage IIb were considered to be anautogenous.

SELECTION. Autogenous strains initially were reared as single families (i.e., usually less than 100 larvae/raft) in 23 × 35 cm pans and were fed 0.5 cc (ca. 0.5 g) of powdered rat chow per pan (ca. 4 mg/larva) per 2 days until pupation. After the F_6 generation, the diet was reduced to 1 mg/larva/2 days to select for females that were autogenous when reared with less food per larva. If autogeny was inherited as a quantitative trait, then individuals expressing autogeny when reared with less food might carry the entire complement of genetic material. Single families were maintained for 7–10 days postemergence, after which the females were isolated individually in 6 dram vials for oviposition. Females that did not oviposit were dissected to determine autogeny status. Parous females which oviposited autogenously were offered a restrained chick as a blood meal source, held until gravid and re-isolated in vials for oviposition. These larger anautogenous egg rafts (usually more than 150 larvae/raft) were reared individually for the next generation. Progeny from single females were maintained as sublines by sibling matings to enhance selection.

In addition to single family selection, autogenous strains from the Br80 and KR83 colonies were selected *en masse* by not offering females a blood meal after February 20, 1984. Larvae were reared at a density of 2–3 rafts per pan and fed ca. 2 mg of rat chow/larva/2 days. Autogeny rates were checked from April through December 1984.

Anautogenous strains were selected as single families with 1 raft (usually more than 150 larvae) per pan (35 × 23 cm), fed an enriched ration of ca. 4 mg/larva/2 days. Single families were held for 7–10 days postemergence and then offered a restrained chick as a blood meal source. Replete females were examined at 100X within 3 hr after feeding when the mechanical pressure of the distended midgut forced the ovaries against relatively transparent abdomenal cuticle. Using this technique, anautogenous females with follicles matured to ≤ Stage IIb were readily distinguishable from autogenous females with follicles matured to ≥ Stage IV. Autogenous and undetermined females were dissected to verify autogeny status. Anautogenous females were held until gravid and isolated for oviposition to produce the subsequent generation. Promising single families within each single female line were maintained as sublines for sibling matings.

CROSSES. After generation F_6, Br80 anautogenous lines were outcrossed among sublines and between female lines to enhance vigor. Families with a low level of autogeny were selected to produce the F_7 generation which was used in subsequent crosses.

During the F_8 generation Br80 anautogenous and KR83 autogenous strains were crossed reciprocally *en masse* to elucidate the mode of inheritance (Experiment 1). F_1 hybrids were crossed reciprocally and backcrossed to parental strains. For all crosses, sexes were separated within 24 hr of emergence, held until 3–5 days old, allowed to mate *en masse* for 3–4 nights in 4 liter carton cages, offered a blood meal, held until gravid, and then isolated for oviposition. The progeny from individual egg rafts were reared on a diet of ca. 4 mg/larva/2 days, held a minimum of 7 days postemergence, and then dissected to determine autogeny rates. Larval density depended upon egg raft size, but generally was less than 150 first instar larvae per pan.

Crosses during F_8 were repeated during December 1984 after 3 months of additional *en masse* selection to confirm the initial results and expand upon the observation that background genotype altered the penetrance of autogeny (Experiment 2). Methods were identical except that crosses were done *en masse* in 1 ft^3 (0.03 m^3) cages with 100 adults per genotype. Three pans of 200 first instar larvae per pan were reared from all egg rafts from each cross hatched *en masse* and were considered a representative sample of all females ovipositing. Larvae were fed a ration of 2 mg/larva/2 days. Adults from each cross were pooled at emergence in 4 liter carton cages, maintained for 5 or more days on 10% sucrose, and a representative sample of up to 150 females per cross dissected to determine follicular development.

RESULTS

SELECTION. Single family selection of the Br80 autogenous strain began with 9 lines, but rapidly decreased to 2 lines by the F_2 generation as lines with families exhibiting low autogeny rates were discarded (Table 1). Autogeny rates of all families combined ranged from 56% for the parental generation to 86% for the F_8 generation. The autogeny rate decreased slightly during the 5th and 6th generations, but then rose after selection pressure was increased by reducing the larval diet in the F_6 generation. Single family lines selected from the KR83 col-

Table 1. Autogeny rates and number of lines remaining for Br80 autogenous and anautogenous strains selected as single families.

	Autogenous		Anautogenous	
Generation	Autogeny % (n)	No. of lines	Autogeny % (n)	No. of lines
P	56 (43)	—	—	—
F_1	53 (392)	9	23 (774)	19
F_2	66 (369)	2	2 (231)	4
F_3	59 (413)	2	3 (326)	2
F_4	56 (346)	2	3 (1178)	2
F_5	36 (137)	1	2 (1587)	2
F_6	40 (188)	1[a]	1 (877)	2
F_7	72 (134)	1[a]	<1 (361)	1
F_8	86 (35)	1[a]	1 (150)	1

[a] Larvae reared on a reduced diet of 1 mg/larva/2 days.

ony failed to survive past the F_3 generation and did not exhibit enhanced autogeny.

En masse selection of the Br80 autogenous strain gradually increased the autogeny rate from 38% on April 12 to 95% on October 10 (Table 2). *En masse* selection of the KR83 autogenous strain increased the autogeny rate from the 84% observed among the colony founders in May 1983 to > 90% from April 12 to August 7, 1984; the autogeny rate then decreased to between 80–90% (Table 2). The decrease in the autogeny rate of the KR83 autogenous strain to 52% on June 5, 1984 was attributed to a faulty timer which failed to extinguish a fluorescent light leaving the colony in constant illumination for a 2–3 week period. Decreases in the penetrance of autogeny due to elongated photoperiod have been described for *Cx. tarsalis* by Harwood (1966).

Single family selection of the Br80 anautogenous strain reduced the autogeny rate from 56% in the parental colony to 2% by the F_2 generation (Table 1). Inbreeding and the discarding of strains with elevated autogeny rates reduced the number of single family lines from 19 in the F_1 generation to 2 by the F_3 generation. Autogeny rates of the 2 most promising lines changed little during selection (Table 3) indicating that changes in the autogeny rate in Table 1 were attributable to the elimination of lines rather than to the changing of the lines genetically. Thus, highly autogenous and anautogenous strains were selected from the same parent Br80 colony.

Table 2. Autogeny rates of the Br80 and KR83 autogenous strains selected *en masse* from February 20 through December 24, 1984.

Date dissected	Br80 % Autogeny (n)	KR83 % Autogeny (n)
April 12	38 (60)	93 (60)
June 5	26 (50)	52 (50)[a]
July 12	67 (52)	100 (55)
July 25	—	99 (70)
August 7	70 (50)	90 (50)
September 14	94 (50)	82 (50)
October 10	95 (43)	87 (60)
November 27	—	59 (75)
December 24	—	88 (119)

[a] Reared under continuous illumination.

Table 3. Autogeny rate of 2 promising lines within the Br80 anautogenous strain for generations F_1 to F_6.

Generation	Line 1 % autogeny (n)	Line 2 % autogeny (n)
F_1	0 (33)	5 (59)
F_2	0 (101)	3 (97)
F_3	3 (307)	4 (24)
F_4	4 (1091)	3 (60)
F_5	2 (1563)	4 (24)
F_6	1 (827)	0 (8)

CROSSES. Outcrosses among sublines within Line 1 of the BR80 anautogenous strain during the F_6 generation did not increase the autogeny rate regardless of genetic distance (Table 4). These results were anticipated, since family sublines were descended from the same anautogenous female. However, the autogeny rate increased to 16.5% when the progeny of 2 lines stemming from different females from the same colony were crossed, even though autogeny rates in both Lines 1 and 2 never exceeded 5% during generations F_1 to F_6 (Table 3). In addition, the autogeny rate increased from 0% (n=50) in generation F_7 to 34% (n=50) in generation F_8, when 3 family sublines within Line 1 were combined *en masse*. However, subsequent selection failed to detect autogeny among 113 females imbibing a blood meal in generation F_9 and among 73 females

Table 4. Crosses during generation F_6 among lines within the Br80 anautogenous strain selected as single families.

Genetic distance[a]	% Autogeny (n)
Siblings	1.0 (100)
1	1.2 (170)
2	2.6 (421)
5	1.6 (385)
6	2.4 (449)
Unrelated[b]	16.5 (97)

[a] 1 to 6 are the number of generations removed from sharing the same parent.
[b] Line 1 and Line 2, Table 3.

dissected 3 months later during December 1984.

Crosses to elucidate the genetics of autogeny were performed using the KR83 autogenous and Br80 anautogenous strains (Table 5). Overall, the autogeny rate of the Br80 anautogenous strain was 1%, while the autogeny rate of the KR83 autogenous strain was 88%. Failure of the KR83 strain to exhibit 100% autogeny was attributed, in part, to the facultative penetrance of the autogenous genotype. In agreement, the progeny of 11 phenotypically anautogenous KR83 females exhibited an overall autogeny rate of 87% (n = 410 females dissected) when reared as single families (range = 71 − 100% autogeny for individual families).

Overall, the results of 7 of 9 crosses done in Experiment 1 were not significantly different (P>0.05) from results obtained in Experiment 2 when tested by contingency χ^2 (Table 5). Autogeny rates in Experiment 1 for crosses 3 and 13 were significantly greater (P<0.01) than autogeny rates in Experiment 2. The autogeny rate obtained in cross 13 in Experiment 2 agreed well with the autogeny rates obtained for the reciprocal cross 11 in both Experiment 1 and 2.

When parental strains were reciprocally crossed (crosses 3 and 5, Table 5), the heterozygous progeny were 88 and 77% autogenous. The autogeny rate of females in cross 4, but not cross 3, was significantly less than the autogeny rate of KR83 females in cross 1 ($\chi^2 = 7.18$, P<0.01). Thus, autogeny rates were higher when the genes for autogeny were inherited through the female.

Reciprocal crosses among the heterozygote F1 progeny produced by crosses 3 and 4, resulted in autogeny rates of 79 and 62%, respectively (crosses 5 and 6, Table 5). The autogeny rate in cross 5 was significantly less than the autogeny rate in cross 1 ($\chi^2 = 6.15$, 0.01 < P < 0.05), but not significantly different from the expected 75% presuming a single dominant autosomal gene mode of inheritance ($\chi^2 = 2.24$, P > 0.05). The 62% autogeny observed in cross 6 was significantly less than cross 5 ($\chi^2 = 25.55$, P<0.01) and the expected 75% ($\chi^2 = 11.41$, P<0.01). Again, autogeny rates were higher for crosses when the gene(s) for autogeny were inherited through the female (i.e., KR/Br).

When both KR/Br and Br/KR hybrids were backcrossed to KR83 males and females (crosses 7 to 10, Table 5), progeny autogeny rates did not vary significantly when tested by heterogeneity chi square ($\chi^2 = 5.54$, P > 0.05). The overall autogeny rate (92%, n = 483) did not differ significantly from the autogeny rate of the KR83 colony in cross 1 ($\chi^2 = 1.89$, P > 0.05).

When hybrid progeny of crosses 3 and 4 were backcrossed to anautogenous Br80 adults (crosses 11 to 14, Table 5), autogeny rates were significantly (P < 0.01) variable within and among experiments (range 21 to 65%). The overall autogeny rate of 41% (n = 835) differed significantly from the expected 50% autogeny rate ($\chi^2 = 25.17$, P < 0.01), but not from 44%, half the autogeny rate observed for the KR83 strain in cross 1 ($\chi^2 = 2.73$, P > 0.05). Lowest autogeny rates in both experiments (24%) were observed when the gene(s) for autogeny was

Table 5. Crosses to elucidate the inheritance of autogeny in *Culex tarsalis*.

Cross	Parental genotypes[1] (♀♀ − ♂♂)	% Autogeny (n) Exp. 1	Exp. 2[2]	Total[3]
1	KR/KR × KR/KR	87 (37)	88 (119)ns	88 (156)**
2	Br/Br × Br/Br	2 (45)	0 (73)ns	1 (118)ns
3	KR/KR × Br/Br	90 (284)	75 (120)**	88 (404)**
4	Br/Br × KR/KR	77 (95)	77 (126)ns	77 (221)**
5	KR/Br × KR/Br	78 (432)	83 (105)ns	79 (537)ns
6	Br/KR × Br/KR	64 (138)	59 (105)ns	62 (243)**
7	KR/KR × Br/KR	97 (63)	88 (105)ns	91 (168)ns
8	KR/KR × KR/Br	—	87 (105)	87 (105)ns
9	Br/KR × KR/KR	—	95 (105)	95 (105)ns
10	KR/Br × KR/KR	—	93 (105)	93 (105)ns
11	Br/Br × Br/KR	24 (100)	24 (150)ns	24 (250)**
12	Br/Br × KR/Br	—	56 (150)	56 (150)ns
13	Br/KR × Br/Br	65 (200)	21 (100)**	50 (300)ns
14	KR/Br × Br/Br	—	37 (135)	37 (135)**

[1] KR = KR83 autogenous strain, Br = Br80 anautogeneous strain; female/male.

[2] Experiment 1 tested against Experiment 2 using contingency chi square, ns P>0.05, *0.01 <P<0.05, **P<0.01.

[3] Total = Experiment 1 and Experiment 2 pooled. Observed percentage autogenous tested against expected percentage autogenous assuming a simple dominant monofactorial autosomal mode of inheritance for autogeny.

inherited through the male and backcrossed to anautogenous females (cross 11).

DISCUSSION

Selection and crossing experiments indicated that autogeny in *Cx. tarsalis* is inherited as a dominant trait which is not sex-linked. O'Meara and Craig (1969) reported that autogeny in *Aedes atropalpus* (Coq.) is controlled by a single dominant autosomal gene. However, in *Cx. tarsalis* autogenous females recurred at a low rate during each generation of selection, even though selection for anautogeny rapidly produced lines with autogeny rates approaching 0%. Similarly, selection for autogeny also failed to produce a homogeneous strain. Failure to attain 100% autogeny in both the KR83 and Br80 autogenous strains was attributed, in part, to facultative penetrance influenced by larval density and food levels. Persisting variability during the selection of both anautogenous and autogenous strains indicated that the inheritance of autogeny in *Cx. tarsalis* may be polyfactorial.

The genetics of autogeny in *Culex pipiens* Linn. appears complex and may be governed by genes on 2 (Spielman 1957) or perhaps 3 (Aslamkhan and Laven 1970) chromosomes. In summarizing crosses performed by Aslamkhan (1963[5]), Laven (1967) proposed a mechanism involving genes on at least 2 of the 3 chromosomes. Low levels of autogeny, such as was exhibited by the Br80 anautogenous strain, were attributed to crossing over among chromosomes I and II and/or II and III. Deletion or replacement of some alleles at loci on chromosome III prevented the expression of 100% autogeny. The absence of similar alleles among the founders of the Br80 or KR83 colonies could have prevented selection from attaining 100% autogeny rates. Further genetic studies are needed to determine if Laven's (1967) model for *Cx. pipiens* may be applicable to the inheritance of autogeny in *Cx. tarsalis.*

Inbred single family lines and *en masse* selected autogenous strains failed to yield 100% homogeneous colonies after 8 generations and 7 months of selection, respectively. Failure to attain 100% autogeny rates also may be attributed to penetrance in some genetically autogenous females. Variable penetrance was demonstrated among the autogeny rates of females from the Knight's Landing colony reared under unstressed and stressed conditions (Reisen et al. 1984).

The expression of autogeny within individual females did not appear to vary as a function of selection pressure. Although fecundity was not estimated, most autogenous females produced more than 50 eggs. Conversely, the fecundity of autogenous females in nature varied temporally as a function of the population autogeny rate (Spadoni et al. 1974).

Although not sex linked, the penetrance of autogeny seemed to be weakened when inherited in a male genotype. Thus, autogeny rates in crosses 4, 6 and 11 where the male was homozygous or heterozygous for autogeny were significantly lower than autogeny rates in crosses 3, 5 and 12–14 where the female was homozygous or heterozygous for autogeny. Perhaps, sex-linked modifying genes may be responsible for enhancing the penetrance of the major autogeny gene(s). Further genetic and physiological studies on autogeny in *Cx. tarsalis* will be required to ascertain the role of sex in the penetrance of autogeny as well as map the linkage group affinities of the major gene for autogeny.

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