

SELECTION-DEPENDENT TRENDS OF AUTOGENY AND BLOOD FEEDING IN AN AUTOGENOUS STRAIN OF *CULEX TARSALIS* (DIPTERA: CULICIDAE)

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ABSTRACT. During successive *en masse* selection of autogeny in *Culex tarsalis* from parental to 20th generation, the autogenous oviposition capacity changed as follows: mean number of egg rafts/female and mean number of eggs/female increased significantly, from 0.11 to 0.56 and from 6.2 to 20.2, respectively; the mean size of the egg rafts decreased significantly, from 56.2 to 36 eggs/raft; autogeny rates increased significantly, from 18.6 to 65.2%; mean number of autogenous follicles/female decreased significantly, from 57.4 to 34; the hatching rate of autogenous egg rafts reduced significantly, from 88.3 to 75.4%; and the feeding rates of the females on a blood meal source during the 7th day postemergence increased significantly, from 62.8 to 71.1%. The autogeny rate determined by ovarian dissection was higher than that indicated by actual oviposition. As the age of females increased during the observed 10-day oviposition period, the mean number of egg rafts/female, the mean number of eggs/female, and the mean number of eggs/raft decreased successively, and scattered "light color" egg rafts became more common. Successive *en masse* selection enhanced autogeny and strengthened the bloodfeeding tendency in this facultative autogenous species.

INTRODUCTION

Autogeny, the development of eggs without ingestion of blood by the adult, is widespread in many mosquito species. Autogenous species or strains can mature 1, 2, or even as many as 4 egg batches in the absence of a blood meal (O'Meara and Lounibos 1981, Gad et al. 1995). Autogeny in mosquitoes is controlled genetically and endocrinologically and is affected by a number of factors, such as larval and adult nutrition, rearing temperature, and photoperiod. Autogeny is important biologically but also in the epidemiology of mosquito-borne disease. The initial blood meal is delayed in obligatory autogenous females and possibly in the facultative females, thereby reducing the number of potentially infective females in the population (Nelson and Milby 1982). Autogenous females oviposit earlier in life, resulting in greater and earlier increase of population growth and eventually the size of the population (Eberle and Reisen 1986, Reisen et al. 1992).

Culex tarsalis Coquillett is an important species, as it serves as vector of arboviral agents to humans and animals, and this species is a major target of surveillance and control efforts in the western United States and elsewhere. Autogeny in this species was reported for the first time by Bellamy and Kardos (1958) and Chao (1958) from a strain in Bakersfield, CA. The facultative autogeny, i.e., genetically autogenous females may engorge during the 1st ovarian cycle if a host is available, was further documented in several studies (Bellamy and Corbet 1973, Nelson and Milby 1982, Eberle and Reisen 1986). The current studies present the trends in autogeny level and bloodfeeding rate in an auto-

nous strain of *Cx. tarsalis* under the *en masse* selection pressure where the females were deprived of blood for 20 generations, and the daily pattern of autogeny level was also noted.

MATERIALS AND METHODS

Mosquito colony handling and autogenous strain selection

A parental strain was started from egg rafts collected at field sites near Oasis, CA, in November 1992. From this parental strain, an autogenous strain was selected *en masse* from egg rafts laid without a blood meal. Larvae were reared in a culture room where 250 1st-instar larvae were placed in a 30 × 19 × 5-cm enamel pan containing 2,000 ml distilled water and were fed dry powder food at the doses of 50, 100, 200, and 150 mg per pan every day for 1st-, 2nd-, 3rd-, and 4th-instar larvae, respectively. The larval food consisted of powdered rat chow and brewer's yeast in the ratio of 3:1. *Culex tarsalis* larvae experience high mortality in the presence of scum on the water surface. The scum was therefore removed from the pans, and water was added every other day to replenish loss due to evaporation. Pupae were removed from the pans as needed and placed in screened cages (23 × 23 × 32 cm), where the adults emerged. Adults were continuously provided with 10% sucrose solution in a jar provided with a dental wick. The colonies were maintained at 26 ± 1°C, 55–65% RH, and a 14:10 h (L:D) photoperiod with 1-h dawn and dusk periods.

The autogenous strain was selected *en masse* continuously by not offering the females a blood source. The selection process went on for 20 generations, and the pattern of autogeny expression and bloodfeeding were determined in the parental, 5th, 10th, 15th, and 20th generations.

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Autogeny level

Oviposition activity: After emergence, females were held in cages where Bermuda grass infusion (Millar et al. 1992) at a dilution of 10% in 100 ml distilled water was used as oviposition attractant in a 120-ml disposable paper cup. The egg rafts laid overnight were collected and counted every day. Dead mosquitoes were removed daily from the cages, and the dead females were counted. The mean number of egg rafts per female and the mean number of eggs per female were calculated as total number of egg rafts/total number of females and total number of eggs/total number of females. The daily trends in number of egg rafts per female and number of eggs per female were calculated as daily total number of egg rafts/daily total number of living females and daily total number of eggs/daily total number of living females. The total and daily mean size of egg rafts were obtained after counting the eggs in all of the egg rafts. Observation of oviposition was conducted until the 10th day after the 1st egg raft was collected. For every test generation, 5 cages with 400–600 females/cage were examined. The means and standard errors were figured based on the data from these 5 cages for the above parameters.

Autogenous rate and autogenous follicles: The autogenous females were maintained on 10% sucrose for 7 days postemergence, after which the females were dissected under a dissecting microscope to determine the autogenous status, to record the autogenous rate, and to count the number of autogenous follicles. Autogenous ovary identification was based on the criterion that females with follicles beyond Christophers' Stage II without ingestion of blood meal were considered to be autogenous. The autogeny rates were determined by the number of females with autogenous follicle(s) divided by the total number of females dissected. Three to 5 batches of females, each batch consisting of about 100 females from each cage, were dissected for the parental, 5th, 10th, 15th, and 20th generations.

Hatching rate of autogenous egg raft

Every day during the oviposition, 2–3 egg rafts deposited daily by the parental, 5th, 10th, 15th, and 20th autogenous generations were randomly selected for determination of hatching rate to explore the possible effect of selection pressure on hatching ability of the eggs. After counting of the eggs in each raft, the egg rafts were placed in a waxed paper cup containing 50 ml distilled water. The larvae were counted after 48 h. The hatching rate was calculated as the number of larvae/total number of eggs. The observation was conducted for 10 successive days after the first oviposition.

Bloodfeeding rate and the relation to ovarian stage

On the 7th day postemergence, the adults with access to sugar solution were allowed to feed overnight on a restrained 1-week-old chick for 12 h. The following morning the bloodfed and nonbloodfed females were counted and dissected to determine the Christophers' ovarian stage. Five groups of about 100 females were dissected in every test generation.

Data analysis

The quantitative data, such as mean number of egg rafts/female, mean number of eggs/egg raft, mean number of eggs/female, and mean number of autogenous follicles/female, were compared with 1-factor ANOVA at the 0.05 level among different test generations. The chi-square test was used to compare the numerical data, such as autogeny rate, hatching rate of egg, and bloodfeeding rate among different test generations. Based on the means of 5 cages in each test generation, the daily trends in oviposition, egg raft size, and hatching were presented as graphs modified by curve fitting.

RESULTS

Autogeny level

Ovipositional activity: Under the pressure of the *en masse* selection, the mean number of egg rafts/female increased significantly, from 0.11 to 0.56; the mean number of eggs/raft decreased significantly, from 56.2 to 36; and mean number of eggs/female increased significantly, from 6.2 to 20.2 (Table 1). Mean number of egg rafts/female, mean number of eggs/raft, and mean number of eggs/female covaried with the increase in female age. During the observed 10 days of oviposition, mean number of egg rafts/female peaked on days 2 and 3 of the oviposition, then decreased gradually (Fig. 1). The number of eggs/raft decreased day by day from the first oviposition and the scattered "light color" egg rafts became more common with the increasing age of females (Fig. 2). The mean number of eggs/female showed the same trend as the mean number of egg rafts/female (Fig. 3).

Autogeny rate and autogenous follicles: Autogeny rates increased significantly, from 18.6 to 65.2%, and mean number of follicles/female decreased significantly, from 57.4 to 34, during the selection from the parental to the 20th generation (Table 1).

Egg hatching rate

The hatching rate of autogenous egg rafts decreased significantly, from 88.3 to 75.4%, during the selection (Table 1). With increasing age of females, the hatching rate of the autogenously oviposited eggs decreased markedly (Fig. 4).

Table 1. Trends in autogeny expression level (mean ± SE) in *Culex tarsalis* on selection from the parental generation to the 20th generation.

Generations	Mean no. of egg rafts/♀	Mean no. of eggs/raft	Mean no. of eggs/♀	% hatch	Mean no. of autogenous follicles/♀	Autogeny rate (%) ¹
0	0.11 ± 0.013 ^{a2}	56.2 ± 8.50 ^a	6.2 ± 1.60 ^a	88.3 ± 0.46 ^a	57.4 ± 3.67 ^a	18.6 ± 1.95 ^a
5	0.24 ± 0.015 ^b	51.9 ± 1.56 ^a	12.3 ± 0.67 ^b	82.1 ± 0.70 ^b	53.2 ± 2.64 ^a	30.7 ± 2.84 ^b
10	0.28 ± 0.012 ^b	51.9 ± 1.56 ^a	14.4 ± 0.99 ^b	85.0 ± 0.55 ^b	53.6 ± 2.59 ^a	35.0 ± 2.82 ^b
15	0.42 ± 0.017 ^c	42.9 ± 1.80 ^b	17.8 ± 0.82 ^c	73.2 ± 0.81 ^c	41.3 ± 1.63 ^b	52.0 ± 1.99 ^c
20	0.56 ± 0.021 ^d	36.0 ± 1.44 ^c	20.2 ± 0.82 ^c	75.4 ± 0.80 ^c	34.0 ± 1.24 ^c	65.2 ± 2.69 ^d

¹ The autogeny rates were determined by the number of females with autogenous follicle(s)/the total number of females dissected.

² For mean number of eggs/female, mean number of egg rafts/female, mean number of eggs/raft and mean number of autogenous follicles/female, the different letters indicate significant differences at the 0.05 level by ANOVA test. For hatching rate and autogenous rate, the different letters indicate significant differences at 0.001 level ($\chi^2 = 11.093-296.441$, $P = 0.001-0.009$), while the same letters indicate no significant differences at 0.05 level ($\chi^2 = 1.141-3.541$, $P = 0.0599-0.2855$) by chi-square test.

Bloodfeeding rate and ovarian stage

The feeding rates of the females on the 7th day postemergence increased significantly, from 62.8 to 71.1%, during the selection from the parental to the 20th generation (Fig. 5). In freshly engorged females, the ovarian stages of the nonbloodfed females were more advanced than those of bloodfed females. Following selection, i.e., in the 15th and 20th generations, the proportion of bloodfed females with ovarian stage V increased markedly (Fig. 5).

DISCUSSION

The expression of autogeny is controlled genetically and regulated by a number of environmental factors. In some species, like *Cx. pipiens*, autogenous and anautogenous forms are segregated by behavioral differences and do not occur in mixed populations even in the same area (Tate and Vincent 1936, Spielman 1964). However, in some facultatively autogenous species, such as *Aedes aegypti* (Trpis 1977) and *Ae. taeniorhynchus* (O'Meara and Edman 1975), the populations may be mixtures of genetically autogenous and anautogenous females in varying proportions according to the geographical locations. The natural populations of *Cx. tarsalis* maintain low-level autogeny and change seasonally (Moore 1963, Spadoni et al. 1974, Reisen et

al. 1992). The selection and crossing experiments by Eberle and Reisen (1986) indicated that autogeny in *Cx. tarsalis* is inherited as a dominant trait that is not sex-linked. The successive selection pressure without a blood meal will eliminate genetically and phenotypically anautogenous individuals.

The techniques for determining autogeny vary in the literature. Some considered the females autogenous when mature eggs were obtained (Kardos 1959), or when follicular development was beyond Christophers' Stage III (Moore 1963, Reisen et al. 1984, Eberle and Reisen 1986, Brust 1991). We use the criterion that females having follicles beyond Christophers' Stage II without ingestion of blood were considered to be autogenous, which is the minimum requirement for the identification of autogenous ovary development. The parameters to describe autogeny level include 1) the autogeny index (the number of eggs oviposited divided by the total number of females) (Trpis 1977, Chambers and Klowden 1994); 2) the autogeny rate or frequency, expressed as the percentage of females with ovaries at or beyond Christophers' Stage III (O'Meara and Edman 1975, Cui 1982, Chambers and Klowden 1994); and 3) fecundity, the number of eggs per autogenous female (O'Meara and Edman 1975, Chambers and

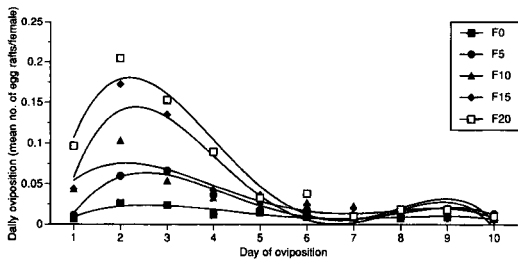


Fig. 1. Daily oviposition by autogenous females in *Culex tarsalis* upon selection from 0 to 20th generation

Fig. 1. Daily oviposition by autogenous females in *Culex tarsalis* on selection from the parental generation to the 20th generation.

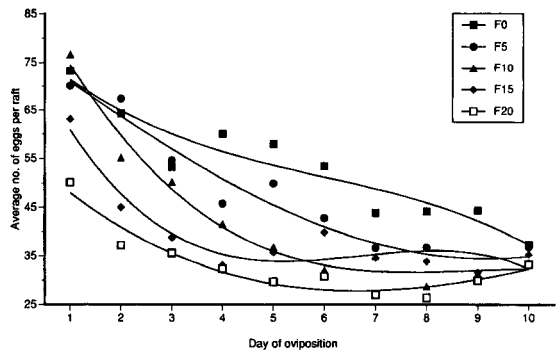


Fig. 2. Daily trend of autogenous egg raft size in *Culex tarsalis* on selection from the parental generation to the 20th generation.

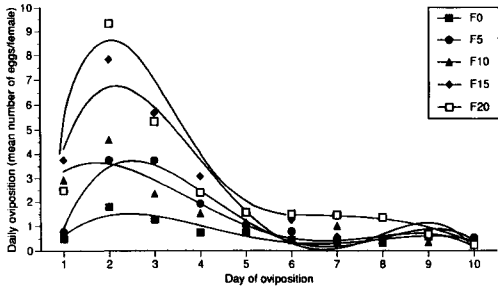


Fig. 3. Daily oviposition by autogenous females in *Culex tarsalis* on selection from the parental generation to the 20th generation.

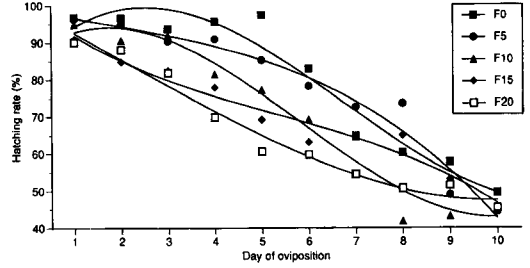


Fig. 4. Daily trend in hatching rates of autogenous egg rafts of *Culex tarsalis* on selection from the parental generation to the 20th generation.

Klowden 1994). In the present research, we used the following parameters to explore the selection-dependent trends in autogeny level: mean number of egg rafts/female, mean number of eggs/raft, mean number of eggs/female, mean number of follicles/female, and autogeny rate (frequency of autogeny). During the selection from parental generation to 20th generation, mean number of egg rafts/female, mean number of eggs/female, mean number of autogenous follicles/female, and autogeny rate increased significantly, while the mean number of eggs/raft decreased significantly. The autogeny rate determined by ovary dissection was higher than that indicated by oviposition or the number of egg rafts per female, which suggested that some of the autogenously gravid females could not oviposit and autogenously developed follicles were reabsorbed. For each observed generation, mean number of eggs/female, mean number of egg rafts/female, and size of egg raft decreased with increasing age of the females. The successive selection pressure without a blood meal made the gene pool governing autogeny purer

by eliminating genetically and phenotypically anautogenous individuals. From the point of view of maintaining the population of the autogenous species, autogenous oviposition rate is probably more meaningful than autogeny rate determined by ovary dissection. Similar selection experiments were also made in this species (Eberle and Reisen 1986) and in *Aedes albopictus* (Chambers and Klowden 1994). In *Cx. tarsalis*, the autogeny rates of all families combined ranged from 56% for the parental generation to 86% for the F₈ generation. However, the selection procedure in those studies was different from that employed in the present study. Briefly, single families were maintained for 7–10 days post-emergence, after which the females were isolated individually for oviposition. Females that did not oviposit were dissected to confirm autogenous status. Parous females that oviposited autogenously were offered a restrained chick as a blood meal source, held until gravid, and reisolated for oviposition. These larger anautogenous egg rafts were reared individually for the next generation. Progeny from single females were maintained as sblings by sibling

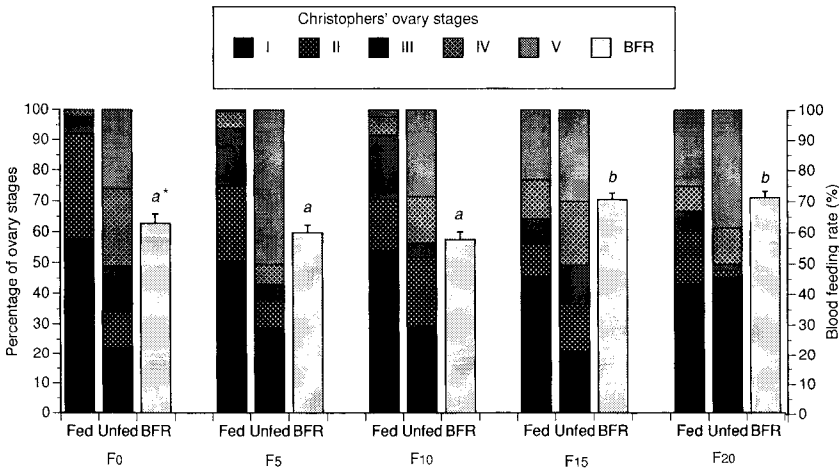


Fig. 5. Bloodfeeding rate (BFR) (percentage \pm SE) of autogenous *Culex tarsalis* on the 7th day postemergence and the ovarian stages of bloodfed and nonbloodfed females. * The different letters at the top of the bars representing bloodfeeding rate indicate significant differences by chi-square test ($P < 0.05$), while the same letters indicate no significant differences ($P > 0.05$).

matings to enhance selection (Eberle and Reisen 1986). In *en masse* selection of autogeny in *Cx. tarsalis* from February 12 to December 24, 1984, the autogeny rates increased from 38 and 84% to 95 and >90%, respectively, in two strains (Eberle and Reisen 1986). In *Ae. albopictus* (Chambers and Klowden 1994), from parental generation to F_{20} , the proportion of autogenous females increased rapidly from 5 to 84%, autogeny index increased from 0.32 to 15.4, and fecundity increased from 6 to 18. In Chambers and Klowden's report, the selection method was also different from ours. All of the autogenous eggs from generation 1 were needed to produce the next generation, females were bloodfed after ovipositing their autogenous eggs, and the resulting offspring were used to determine the percentage of autogeny and numbers of eggs for generations 5–15 (Chambers and Klowden 1994). Consequently, the experimental results of selection on autogeny shared both the selection-dependent increase in autogeny level and the range of changes in autogeny level, which can be attributed to the differences in species or strain origin, selection procedures, autogeny identification criteria, etc.

For the facultatively autogenous species including *Cx. tarsalis* (Bellamy and Corbet 1973, Reisen and Milby 1987), the relatively low fecundity expressed as autogeny could be an alternative strategy for maintaining the population when the hosts are scarce. The facultative bloodfeeding would seem to provide gonotrophic flexibility for dealing with environments under this situation. The combined autogenous and anautogenous ovarian development in *Cx. tarsalis* indicated the optional requirement for blood meal in the first gonotrophic cycle (Bellamy and Corbet 1973). Mark-release-recapture technique revealed that autogenous *Cx. tarsalis* did not seek blood until after they had oviposited the first batch of eggs (Nelson and Milby 1982). However, Reisen and Milby (1987) reported that autogenous females of *Cx. tarsalis* readily took blood meals from restrained chickens if ovarian development had not progressed to Christophers' Stage III. Here we even found that some females with ovarian development at Stage V still readily fed on restrained chicks. The bloodfeeding rate on day 7 postemergence tended to increase under the selection pressure.

It is thus evident that the successive *en masse* selection of an autogenous strain enhanced autogeny expression level and strengthened the bloodfeeding tendency in this facultative autogenous species.

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REFERENCES CITED

Bellamy, R. E. and P. S. Corbet. 1973. Combined autogenous and anautogenous ovarian development in indi-

- viduals *Culex tarsalis* Coq. (Diptera: Culicidae). Bull. Entomol. Res. 63:335–346.
- Bellamy, R. E. and E. H. Kardos. 1958. A strain of *Culex tarsalis* Coq. reproducing without blood meal. Mosq. News 18:132–134.
- Brust, R. A. 1991. Environmental regulation of autogeny in *Culex tarsalis* (Diptera: Culicidae) from Manitoba, Canada. J. Med. Entomol. 28:847–853.
- Chambers, G. M. and M. J. Klowden. 1994. Nutritional reserves of autogenous and anautogenous selected strains of *Aedes albopictus*. J. Med. Entomol. 31:554–560.
- Chao, J. 1958. An autogenous strain of *Culex tarsalis*. Coq. Mosq. News 18:134–136.
- Cui, K. L. 1982. The autogeny of *Aedes albopictus* in the Guangzhou area. Acta Entomol. Sin. 25:256–259.
- Eberle, M. W. and W. K. Reisen. 1986. Studies on autogeny in *Culex tarsalis*. 1. Selection and genetic experiments. J. Am. Mosq. Control Assoc. 2:38–43.
- Gad, A. M., A. N. Hassan and H. A. Farid. 1995. Preimaginal vitellogenic development and repeated autogeny in *Culex pusillus* (Diptera: Culicidae) from Egypt. J. Med. Entomol. 32:384–386.
- Kardos, E. H. 1959. The effect of larval nutritional level on the development of autogeny in colony *Culex tarsalis*. Coq. Proc. Calif. Mosq. Control Assoc. 27:71–72.
- Millar, J. G., J. D. Chaney and M. S. Mulla. 1992. Identification of oviposition attractants for *Culex quinquefasciatus* from fermented Bermuda grass infusions. J. Am. Mosq. Control Assoc. 8:11–17.
- Moore, C. G. 1963. Seasonal variation in autogeny in *Culex tarsalis* Coq. in northern California. Mosq. News 23:238–241.
- Nelson, R. L. and M. M. Milby. 1982. Autogeny and blood-feeding by *Culex tarsalis* (Diptera: Culicidae) and the interval between oviposition and feeding. Can. Entomol. 114:515–521.
- O'Meara, G. F. and J. D. Edman. 1975. Autogenous egg production in the salt-marsh mosquito, *Aedes taeniorhynchus*. Biol. Bull. 149:384–396.
- O'Meara, G. F. and L. P. Lounibos. 1981. Reproductive maturation in the pitcher-plant mosquito, *Wyeomyia smithii*. Physiol. Entomol. 6:437–443.
- Reisen, W. K. and M. M. Milby. 1987. Studies on autogeny in *Culex tarsalis*. 3. Life table attributes of autogenous and anautogenous strains under laboratory conditions. J. Am. Mosq. Control Assoc. 3:619–625.
- Reisen, W. K., M. M. Milby and M. E. Bock. 1984. The effects of immature stress on selected events in the life history of *Culex tarsalis*. Mosq. News 44:385–395.
- Reisen, W. K., M. M. Milby and R. P. Meyer. 1992. Population dynamics of adult *Culex* mosquitoes (Diptera: Culicidae) along the Kern River, Kern County, California, in 1990. J. Med. Entomol. 29:531–543.
- Spadoni, R. D., R. L. Nelson and W. C. Reeves. 1974. Seasonal occurrence, egg production, and blood-feeding activity of autogenous *Culex tarsalis*. Ann. Entomol. Soc. Am. 67:895–902.
- Spielman, A. 1964. Studies on autogeny in *Culex pipiens* population in nature. I. Reproductive isolation between autogenous and anautogenous population. Am. J. Hyg. 80:175–183.
- Tate, P. and M. Vincent. 1936. The biology of autogenous and anautogenous races of *Culex pipiens* L. (Diptera: Culicidae). Parasitology 28:115–145.
- Trpis, M. 1977. Autogeny in diverse populations of *Aedes aegypti* from East Africa. Tropenmed. Parasitol. 28:77–82.