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FECUNDITY, FERTILITY, AND DEVELOPMENTAL STAGE SURVIVORSHIP OF *ERETMAPODITES QUINQUEVITTATUS*

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ABSTRACT. This laboratory study analyzed fecundity, fertility (percentage hatched), and developmental stage survivorship of *Eretmapodites quinquevittatus.* Fecundity for females averaged 190.4 eggs, whereas paired (one male/female pair) females averaged 100.5 eggs. Fertility for autogenous eggs from paired females was 84.4%, which was significantly greater than percentage hatched of anautogenous eggs (67.3%). Percentage hatched of autogenous eggs from experimental groups (25 males/25 females) was 87.8%, whereas percentage hatched of anautogenous eggs was 74.5%. Developmental stage survivorship was significantly different for every stage of development of progeny from autogenous eggs, except the egg stage, when comparing experimental groups with pairs. Developmental stage survivorship of progeny from anautogenous eggs was significantly different only at the egg stage and 1st instar when comparing experimental groups and pairs.

Eretmapodites quinquevittatus Theobald occurs throughout subsaharan Africa and Madagascar and has been implicated in the transmission of various arboviruses (Macnamara 1953, McIntosh et al. 1961, Worth et al. 1961, Ardoin and Simpson 1965, Serie et al. 1968, Brottes et al. 1969). In regard to the reproductive biology of this species, preliminary observations indicate that *Er. quinquevittatus* females are autogenous and refuse to bloodfeed before laying a batch of eggs, with an average production of 52 autogenous eggs per female (Hartberg and Gerberg 1970). Under laboratory conditions, the size of the container and the number of *Er. quinquevittatus* mating pairs may affect the average number of autogenous eggs produced by females of this species (M. A. Stanley and W. K. Hartberg, unpublished data). For example, 30 out of 40 male/female pairs held individually in 1-pint (0.6-liter) containers deposited 782 eggs or an average of 26.1 autogenous eggs per female at 25°C and 80% RH. In contrast, 35 out of 40 male/female pairs held in 1-gallon (4.3-liter) containers deposited 1,229 eggs for an average of 35.1 autogenous eggs per female. Stanley and Hartberg (unpublished data) believed that females of *Er. quinquevittatus* must be fertilized by a male before they lay eggs.

Knowledge about any mosquito species' reproductive biology is key to better understanding the bionomics and population dynamics of the species and the potential role the species might play in the vectoring of disease agents. For this reason, a study was initiated to expand our knowledge of the reproductive biology of *Er. quinquevittatus* as it relates to the fecundity and fertility rates for this species and to provide new information on the survivorship of *Er. quinquevittatus* during different stages of its developmental cycle. Fecundity was defined as the potential reproductive capacity of *Er. quinquevittatus* or the quantity of gametes produced per female or experimental group of females (Clements 1992). Fertility was defined as the actual reproductive performance of the species or the total number of viable offspring produced by individual females or by experimental groups of females (Clements 1992). These reproductive parameters were evaluated for *Er. quinquevittatus* in terms of whether the eggs were produced autogenously or anautogenously and whether the eggs were produced by females held singly with one male or by females held as an experimental group of 25 females with an equal number of males. We believe any differences in fecundity and fertility found for these 2 different sized containers might help in developing better laboratory procedures. We found from earlier research that males of *Er. quinquevittatus* live longer than other species of males and live as long as females of *Er. quinquevittatus* and females of other species (Helleck et al. 1993). This might indicate that *Er. quinquevittatus* needs more males in an artificial or natural population for females to produce optimally.

In the portion of the study focusing on developmental stage survivorship for *Er. quinquevittatus,* consideration was given to larvae hatching from autogenously produced eggs and larvae hatching from anautogenously produced eggs. This aspect of the study also examined the effects of larval and pupal densities on the developmental survivorship of *Er. quinquevittatus.* Eggs formed autogenously and anautogenously differ in whether a blood meal preceded the formation of eggs. It would be interesting to note if there are any differences in development of larvae/pupae when comparing the 2 types of eggs under ideal conditions. We believe it would be beneficial to know if, under ideal conditions,

the number (density) of larvae/pupae inhabiting a given aquatic medium might influence development.

Laboratory cultures of *Er. quinquevittatus* were maintained in a walk-in environmental chamber set at 25°C, 80 ± 5% RH, and a 14:10 h (light:dark) photoperiod. Three adult experimental groups (25 adult males and 25 adult females) were held in cylindrical gallon-sized cages, and 50 adult pairs (one male and one female each) were held in cylindrical pint-sized cages. Both types of cages were covered with nylon netting. Female mosquitoes were allowed to lay their eggs on moist, bleached, paper towel (oviposition or egg papers) lining the inside walls of 250-ml beakers or half-pint Mason jars (279.3 ml) in the gallon cages or lining the inside walls of cylindrical, glass, shell vials (42.5 ml) in the pint cages. Mason jars or 250-ml beakers contained 150–200 ml of tap water and small glass vials contained 40 ml of tap water to keep the egg papers moist and provide a medium for 1st-instar larvae after hatching.

To establish experimental groups and pairs, eggs were obtained from the EQ-MIXED colony of *Er. quinquevittatus* maintained at the Medical Entomology Research Laboratory at Baylor University. This colony was established by W. K. Hartberg from larvae collected from a tree hole in the Kisutu section of Dar es Salaam, Tanzania, in July 1969 and is maintained in our laboratory as reported by Hartberg and Gerberg (1971). Eggs obtained were a mixture of autogenous and anautogenous eggs. Eggs were not stored. Eggs hatched ≈48 h after oviposition, larvae were cultured, and resulting pupae were sexed and placed in 1-pint emergence cups (≤10/cup). Emerging adults were randomly assigned to experimental group and pair cages.

Eggs collected from experimental groups or pairs were allowed to hatch in oviposition containers. Larvae were transferred to Nalgene™ pans or were allowed to develop in oviposition containers. Progeny from egg papers produced by females in gallon cages with >30 eggs were reared in Nalgene pans (32 × 26 × 6 cm or 26 × 16 × 6 cm). These egg papers usually contained ≥100 eggs. Progeny from egg papers produced by females in pint cages with <30 eggs were reared in 250-ml beakers or Mason jars. These egg papers usually contained <20 eggs. Because of this large difference in egg numbers from gallon-cage females and pair-cage females, survivorship was compared for differences in larval development in large and small groups. Pupae were transferred to pint-sized emergence cups where adults emerged (≤10/cup).

During their development, larvae were fed a slurry of Tetramin® fish food placed at the bottom of Nalgene pans, beakers or Mason jars. Each container of larvae was provided approximately 0.25 ml of food daily depending on the number of larvae in a container. Adults were provided sugar cubes for a carbohydrate source, and females were provided anesthetized mice for blood meals (animal use protocols were approved by the Animal Care and Use Committee at Baylor University, which follows the guidelines of the PHS/NIH). Blood meals were given when autogenous egg production ceased. Twelve hours after anautogenous egg production subsided, another blood meal was given. Thus, blood meals were provided every 4–5 days.

Egg papers were collected every 6 h, from experimental group and pair mosquito containers, and replaced with new papers. Approximately 72 h later, all eggs were counted (fecundity) and hatched eggs were counted (fertility). First-instar larvae hatching from egg papers numbering >30 (experimental group/gallon cages) were transferred to large Nalgene pans. First-instar larvae hatching from egg papers numbering <30 (pair/pint cages) were left to develop in the containers in which they hatched. Progeny that successfully hatched were counted as they progressed through the developmental stages (survivorship). As each group of larvae/pupae progressed to the next developmental stage, they were counted by documenting each individual larva/pupa as it was transferred to another container. This occurred at the end of every developmental stage except the adult stage. Adults were counted and discarded.

Autogenous and anautogenous eggs were analyzed for differences in fertility by one-way ANOVA (arcsine transformation) for pair data only. Progeny from autogenous and anautogenous eggs were analyzed for differences in developmental stage survivorship by the chi-square goodness-of-fit test. Eggs from experimental group and pair females were analyzed for differences in fecundity by comparing average number of eggs laid per female. Eggs from experimental group and single pair females were analyzed for differences in fertility by comparison of percentage hatched values. Experimental group and single pair female progeny were analyzed for differences in developmental stage survivorship by the chi-square goodness-of-fit test. Alpha and power for the statistical tests were 0.05 and 0.80, respectively.

Fecundity for females of *Er. quinquevittatus* was greater when females were held in experimental groups (Table 1). Autogenous and anautogenous egg-laying averages per female, and total numbers of eggs laid, were greater for females held in experimental groups (Table 1). Females laying eggs in experimental groups aver-

Table 1. Fecundity (eggs laid) and fertility (% hatched) of females of *Eretmapodites quinquevittatus* laying autogenous and anautogenous eggs in experimental groups[1] or pairs[2]. Adjusted fecundity (eggs laid/female adj.) provides extrapolated values for experimental group females if the same percentage of females had laid eggs in experimental groups as did in pairs.

	Experimental groups of females		Mating pair females	
	Autogenous	Anautogenous	Autogenous	Anautogenous
Eggs laid	3,994	10,280	1,279	1,792
Eggs hatched	3,508	7,660	1,080	1,206
% hatched	87.8	74.5	84.4	67.3
Eggs laid/female	53.3	137.1	36.5 ± 3.7	64 ± 11.0
Eggs laid/female adj.	64.5	244.8		

[1] Three 1-gallon cages or 75 females.
[2] Fifty pair cages or 50 females.

aged 53.3 autogenous eggs and 137.1 anautogenous eggs, whereas females laying eggs in pairs averaged 36.5 autogenous and 64 anautogenous eggs. Average fecundity for females held in experimental groups was 190.4 eggs, whereas average fecundity for females held in pairs was 100.5 eggs.

These findings are consistent with the findings of M. A. Stanley and W. K. Hartberg (unpublished data) for autogenous eggs laid by females of *Er. quinquevittatus*. They found that females laid fewer eggs when held in smaller containers. Females in M. A. Stanley and W. K. Hartberg's study averaged 26.1 autogenous eggs as compared with our average of 36.5 autogenous eggs in individual pair cages (pint cages). Also, females in that study averaged 35.1 autogenous eggs compared with our average of 53.3 autogenous eggs in experimental groups (gallon cages). This latter average is comparable with that (52 autogenous eggs) reported by Hartberg and Gerberg (1970).

There may be a greater difference in fecundity between experimental group and pair females because we do not know how many of the experimental group females actually laid eggs. We know that 35 of 50 (70%) paired females laid autogenous eggs and 28 of 50 (56%) laid anautogenous eggs. Thus, if only 70% of the 75 experimental group females (53 females) laid autogenous eggs, the average number of eggs per female would be 74.5 eggs rather than 53.3 eggs. If only 56% of the 75 experimental group females (42 females) laid anautogenous eggs, the average number of eggs laid per female would be 244.8 eggs rather than 137.1 eggs. Our study supports the observations of M. A. Stanley and W. K. Hartberg (unpublished data) that there is clearly a difference in autogenous and anautogenous egg production when females mate and lay eggs in larger containers.

This difference may be attributed to the number of males in a cage. In experimental groups, more males were available to inseminate because only one male was available per pair. It is believed *Er. quinquevittatus* females must be inseminated before they will oviposit (Hartberg and Gerberg 1970). A. S. Arrington and W. K. Hartberg (unpublished data) estimate that one male *Er. quinquevittatus* can inseminate up to 9 females.

Fertility (percentage hatched) for females was greater for autogenous eggs in experimental groups (gallon cages) or pairs (pint cages) (Table 1). Percentage hatched of autogenous eggs for pairs was 84.4%, and percentage hatched for anautogenous eggs from pairs was 67.3%. There was a significant difference between the percentage hatched of autogenous and anautogenous eggs laid by females in pairs ($P < 0.001$, $F = 13.4$). Percentage hatched of autogenous eggs laid by females of 3 experimental groups was 87.8%, and percentage hatched for anautogenous eggs laid by females of the same 3 experimental groups was 74.5%. Although the experimental group percentage hatched values seem to be different, they could not be statistically tested because the measure of variation would be based on only 3 occurrences.

It appears that the size of container and the number of females in the container had no effect on percentage hatched values because autogenous percentage hatched for experimental groups and pairs (87.8 and 84.4%, respectively) and anautogenous percentage hatched for experimental groups and pairs (74.5 and 67.3%, respectively) were not different. Statistical analysis was not performed because of differences in sample size (3 gallon cages vs. 50 pint cages). There was a statistical difference in percentage hatched when comparing autogenous eggs with anautogenous eggs from pairs ($P < 0.001$, $F =$

Table 2. Survivorship percentage for each developmental stage of progeny from autogenous and anautogenous eggs produced by females of *Eretmapodites quinquevittatus* held in one experimental group[1] or pairs[2] in the laboratory.

	Experimental group of females		Mating pair females	
	Autogenous	Anautogenous	Autogenous	Anautogenous
Egg–1st instar	80.6	82.0	86.0 ± 6.3	70.6 ± 6.8
1st–2nd instar	89.6	78.8	88.8 ± 4.7	94.6 ± 6.1
2nd–3rd instar	99.2	96.0	75.0 ± 5.4	98.7 ± 6.2
3rd–4th instar	99.1	98.1	73.4 ± 5.8	99.6 ± 6.2
4th instar–pupa	91.6	93.8	65.5 ± 6.2	95.7 ± 6.1
Pupa–adult	89.4	95.5	65.0 ± 7.4	90.4 ± 5.7

[1] All progeny produced from 1-gallon cage consisting of 25 males and 25 females.
[2] All progeny produced from 25 pair cages consisting of 25 male/female pairs.

13.4). Autogenous eggs hatched at rates of 87.8 and 84.4% as compared with anautogenous egg hatching rates of 74.5 and 67.3%. This occurrence might be attributed to autogenous eggs being laid without a preceding blood meal and anautogenous eggs being laid after blood meals that may have varied in quantity and quality.

Survivorship (percentage survived) during development of progeny from autogenous eggs from the one experimental group analyzed was significantly greater than survivorship of progeny from autogenous eggs from single pairs at every stage ($P < 0.01$) except the egg stage ($P > 0.05$; Table 2). Survivorship for anautogenous progeny from experimental group females was significantly greater for the egg stage when compared with anautogenous progeny produced by single pair females ($P < 0.01$; Table 2). Survivorship for anautogenous progeny from the experimental group was significantly less for the 1st instar when compared with anautogenous progeny produced by pairs ($P < 0.01$). Survivorship rates for autogenous and anautogenous progeny produced by females held in the experimental group were significantly different for every stage ($P < 0.01$) except the egg stage ($P > 0.05$). Survivorship rates for autogenous and anautogenous progeny produced by single pair females were significantly different for every stage of development ($P < 0.01$).

Survivorship for the developmental stages was greater for autogenous progeny from the experimental group when compared with autogenous progeny from pairs after the egg stage (Table 2). Survivorship for anautogenous progeny was higher for the experimental group at the egg stage, less at the 1st instar, and not significantly different for any other stages when compared with anautogenous progeny from pairs. Because progeny from the experimental group were cultured in pans with ≥100 larvae and progeny from pairs were cultured in containers with far fewer larvae (<30), differences in density during development could be a factor. Progeny produced by single pair females tended to have lower survivorship throughout development when compared with the progeny from the experimental group females.

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