

A MECHANICAL DEVICE FOR SEPARATING THE DEVELOPMENTAL STAGES, SEXES AND SPECIES OF MOSQUITOES¹

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In large scale production of mosquitoes for investigational purposes, the removal of pupae from the other developmental stages in the rearing containers has often proved a monotonous and time-consuming task. The present paper describes a relatively simple mechanism which performs this operation rapidly and efficiently. Other applications of the apparatus, such as the separation of different species and the separation of the two sexes of a given species are also described.

While the examples noted are confined to the use of the separator in mosquito production, it is felt that wider application may be found for other aquatic organisms in many ecological studies.

APPARATUS. The separator device (Figure 1) consists of a funnel in which the contents of an aquatic insect culture are poured. The culture leaves the funnel through a flattened metal spout and impinging on the upper surface of a base sheet of plate glass (Figure 1, part no. 7), enters a wedge-shaped space between the base sheet and a covering sheet of plate glass (Figure 1, part no. 6). The opening at the upper ends of the glass plates is held constant by metal spacers while at the lower ends of the plates the opening can be precisely regulated with an adjusting screw knob. The lower opening is adjusted so that the larger organisms in the culture are retained between the glass plates while the rest of the culture drains into a receiving container below. Metal guide strips along the sides of the glass plates confine the water and direct its flow into the receiving container. When the retained organisms accumulate in sufficient numbers to impede the water flow, they can be

flushed into a separate container by turning the adjusting screw knob to open the lower ends of the glass plates.

RESULTS

GENERAL OPERATION. A suitable adjustment of the separator can be made quite rapidly by visual inspection. With the lower opening almost closed, the various larval instars and pupae of mosquitoes collect in a series of separate bands with the different instars or pupae at various heights between the glass plates and the smallest forms in the lowest band. With gradual increase of the lower opening, the smaller organisms are flushed out and the bands of larger organisms descend between the glass plates until the desired stage represents the lowest band. If the stage to be separated is not the largest, e.g., the removal of the 4th-instar larvae from younger larvae and pupae, the first adjustment releases the young larvae and a second adjustment releases the 4th-instar larvae while the pupae are retained in the separator. Once the desired settings have been determined, calibrations on the adjustment screw knob permit rapid duplication of any given setting. With 16 divisions on the knob, each division changes the width of the lower opening by 0.0014 inch on the model described.

SEPARATION OF MOSQUITO PUPAE. In removing pupae from mixed culture of larvae and pupae, two factors have influenced the speed of operations: (a) the frequency with which flushing is necessary and (b) the rate of water flow through the separator. From 800 to 900 pupae can be collected in the separator before large larvae are blocked and flushing becomes necessary. At settings required to retain mosquito pupae, one liter of water takes about 45 seconds to pass through the separator. In

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ordinary insectary production with cultures ranging from 5 to 100 pupae per liter of water, the separation of 1000 pupae requires about 20 minutes. In contrast, the picking of pupae from similar cultures with small screen-wire spoons by skilled operators requires about 90 minutes per thousand pupae.

Tests have been made to determine any possible injury to the pupae processed through the separator. Emergence and survival rates of adult mosquitoes from pupae collected with the separator and those collected with the screen-wire spoon technique showed no significant differences.

SEPARATION OF MOSQUITO SPECIES. In field collections of mosquito larvae and pupae, other invertebrate predators or competitors often are taken inadvertently. The separator has been useful in rapidly excluding most of these forms where a size differential existed between the organisms and the desired mosquito specimens.

In certain instances, the separator has been employed to divide mixed cultures of two mosquito species into fractions containing essentially only a single species. A mixed culture of *Aedes sollicitans* and *A. taeniorhynchus* pupae were processed

through the separator by widening the lower opening stepwise and flushing the separator between adjustments to remove the pupae in successive size groups. Each size group was then caged and the emerging adults identified (Table 1). Over a 14-day period, the 4500 pupae studied showed a distribution by species and sex of 34.5 percent male and 38.3 percent female *A. taeniorhynchus* and 13.7 percent male and 13.5 percent female *A. sollicitans*. In separating the two species, the separator could be adjusted to a slit width of 0.057 inch and the pupae which passed through the slit would comprise at least 94 percent *A. taeniorhynchus*. This sample would contain 97 and 88 percent of the available male and female *A. taeniorhynchus* pupae. The separator could be adjusted then to an opening of 0.060 inch, and the next sample flushed would contain 2 and 11 percent of the total male and female *A. taeniorhynchus* pupae mixed with 22 and 16 percent of the total male and female *A. sollicitans* pupae. The separator could be opened fully and the remaining pupae would consist of at least 96 percent *A. sollicitans*. This sample would have 70 and 78 percent of the available male and female pupae of *A. sollicitans*.

TABLE 1.—Partial separation of a mixed culture^a of *Aedes sollicitans* and *Aedes taeniorhynchus* pupae by successive settings of the pupae separator

Width of opening	Species composition (percent) of sample passing opening				Cumulative percent of population removed			
	<i>A. taen.</i>		<i>A. soll.</i>		<i>A. taen.</i>		<i>A. soll.</i>	
	M	F	M	F	M	F	M	F
.045"	90	10	—	—	4	0.5	—	—
.048"	78.5	20	—	1.5	13.5	2.5	—	0.5
.051"	71	28	0.5	0.5	50	15	0.5	1.0
.054"	46	53	0.5	0.5	83	50	2.0	2.5
.057"	24	70	4	2	97.5	88	8.0	6.0
.060"	9	40	30	21	99	99	30	22
.062"	1	3	54	42	99.5	99.5	66	50
.065"	0.5	1	47	51.5	100	100	88	74
.068"	—	—	35	65	—	—	98.5	94.5
.071"	—	—	23	77	—	—	100	99
.074"	—	—	—	100	—	—	—	100

^aCulture composition 34.5 percent male and 38.3 percent female *A. taeniorhynchus* and 13.7 percent male and 13.5 percent female *A. sollicitans*.

SEPARATION OF MOSQUITO SEXES. In certain research work the investigator may have interest in only one sex of the adult insects. The separator has been employed in the sex separation of mosquito pupae with greater or less degrees of success depending upon the species involved. The results of sex separation of the pupae from insectary populations having essentially equal numbers of each sex are shown in Table 2. The effectiveness of the separa-

cent of all the males in the population under separation. Based on these factors, the species tested are listed in order of increasing effectiveness of the separator in dividing the sexes of the species. The best sex separation occurs in those species with the smallest pupae and the most rapid development.

SUMMARY. A mechanical device is described which permits the rapid and accurate separation of mosquito pupae from

TABLE 2.—Partial separation of male and female pupae of various species of mosquitoes by successive settings of the pupa separator

Species	Sex sample desired	Slit width (inch)	Sex division			
			% in sample		% of total culture	
			Male	Female	Male	Female
<i>Aedes sollicitans</i>	Male	.060	59	41	31	22
	Mixed	.065	53	47	57	52
	Female	.065+	33	67	12	26
<i>Anopheles quadrimaculatus</i>	Male	.047	77	23	55	11
	Mixed	.049	36	64	35	42
	Female	.049+	12	88	10	47
<i>Aedes taeniorhynchus</i>	Male	.045	90	10	85	10
	Mixed	.047	32	68	13	28
	Female	.047+	3	97	2	62
<i>Culex quinquefasciatus</i>	Male	.030	95	5	91	9
	Mixed	.042	59	41	8	13
	Female	.042+	10	90	1	78
<i>Aedes aegypti</i>	Male	.040	98	2	87	2
	Mixed	.043	44	56	10	15
	Female	.043+	5	95	3	83

tion can be based on two values: (a) the percentage of the desired sex in a sample obtained with a given slit setting, and (b) the percentage of the total available members of a given sex contained in the sample. For example, the male sample of *Aedes taeniorhynchus* obtained through a slit opening of 0.045 inch (Table 2) contained 90 percent males mixed with 10 percent females. These males represented 85 per-

cent of the larvae in insectary culture containers. The separator has also been useful in separating species commonly found together in field collections such as *Aedes sollicitans* and *Aedes taeniorhynchus*. Further applications of the separator have been found in dividing the pupae of certain mosquito species into male, mixed, and female subsamples.