A device for the rapid separation of male and female mosquito pupae

V. P. SHARMA,[1] R. S. PATTERSON,[2] & H. R. FORD [3]

Abstract

A new device for separating male and female C. p. fatigans *is described. The device is particularly adaptable for the separation of large numbers of pupae without mechanical injury. It also appears to be suitable for use with other species of mosquito.*

The separation of male and female mosquitos is necessary in many experimental investigations,—e.g. genetic studies, insecticide testing, and mass releases of sterile males. Since virgin males or females are usually required, the separation must preferably be done in the pupal stage. This is convenient as pupae are more easily handled and in many species the sex of the pupae can be determined by their size.

Separating devices have been described by Fay & Morlan (1959), McCray (1961), and Gerberg et al. (1969). However, these separators suffer from the disadvantage that they must be flushed periodically, or female pupae will block the openings and prevent the passage of male pupae. Furthermore, if the water pressure used in these devices is too high the pupae may be injured.

Description of the separator and method of use

The pupal separator described below uses not only the principle of size difference between the sexes, as do all the other devices, but also the oxygen requirement of the pupae. The male and female pupae are submerged in water under a nylon grid. In attempting to rise to the surface for their normal oxygen requirements, the pupae repeatedly attempt to pass through the grid. The spacing between the threads determines the maximum size of pupa that may pass through; larger pupae are trapped under the grid. No force is applied to the pupae and therefore there is no mortality resulting from mechanical injury. Furthermore, small pupae that are blocked by large pupae in one place can dive and come up elsewhere, and thus are not trapped as in the other devices.

The design of the separator is very simple. It consists of a grid (Fig. 1) of evenly spaced parallel nylon threads held under tension by the side bars of an aluminium frame. On two opposite sides of the frame there are aluminium strips with slots cut at equal distances for spacing the nylon thread. The inside dimensions of the frame are 97×102 mm and the diameter of the nylon thread is 0.81 mm. The total number of nylon threads, and hence the spacing between threads, varies from frame to frame; for example, if 53 threads are present the opening is 1.02 mm and with 56 threads it will have an opening of 0.92 mm. Five grids with distances between the threads of 0.89 mm, 0.92 mm, 0.95 mm, 0.98 mm and 1.02 mm were evaluated. An aluminium tray of the same dimensions as the grid holds the pupae (Fig. 2).

Selection of the grid depends on the pupal size and the accuracy of sex separation desired. Preliminary checks of the pupae for accuracy of sex separation can be easily made by a technician, using a dissecting microscope. With *C. p. fatigans* larvae reared on a standard diet of ground dog biscuits and brewer's yeast it was found that the average distance across the back of the cephalothorax of the female was 1.295 mm, whereas that of the male was 0.962 mm.

Once the correct grid size has been determined, the pupae to be sexed are transferred to the tray and placed in a plastic bowl (Fig. 3). The tray is then covered with the grid (Fig. 4) and filled with water. The small (mostly male) pupae pass through the grid (Fig. 5) and rise to the surface. The pupae flow out of the tub at the overflow spout and are collected on a nylon net (Fig. 6). After the collection of the male pupae in the net, the grid is removed and the remaining pupae (mostly females) rise to the surface and are collected. Separation does not take more than 5 minutes for several thousand pupae with this small grid.

[1] Senior Scientist, Indian Council for Medical Research, New Delhi, India.

[2] Entomologist, WHO Research Unit on Genetic Control of Mosquitos, New Delhi.

[3] Entomology Research Division, US Department of Agriculture Research Service, Gainesville, Fla., USA.

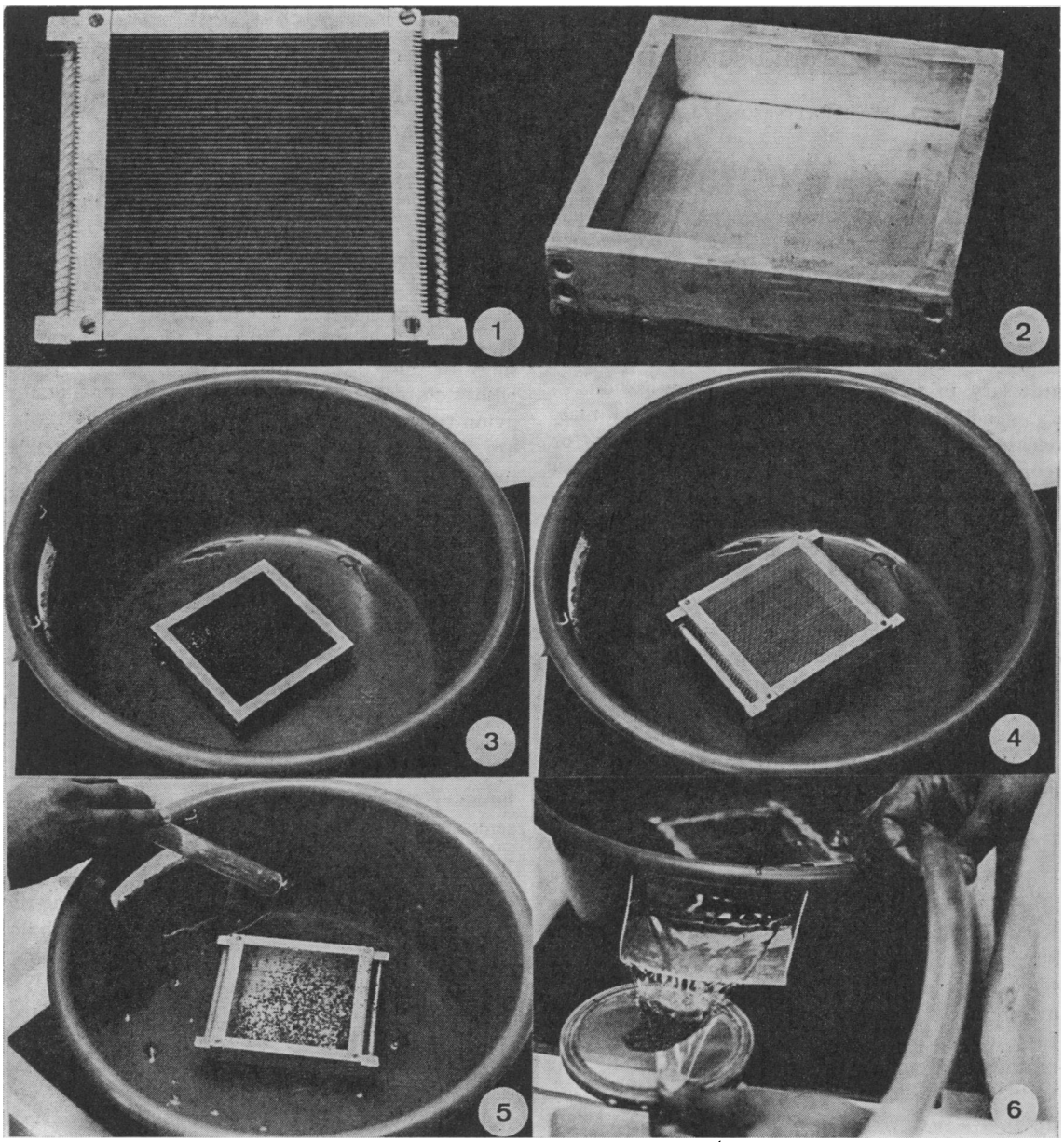

Fig. 1. Grid for separation of pupae, consisting of an aluminium frame with an approximately 10 cm square opening (inside dimensions) crossed by evenly spaced nylon threads under tension.

Fig. 2. Aluminium tray for holding pupae.

Fig. 3. Tray with pupae in plastic bowl.

Fig. 4. Tray holding pupae covered with grid.

Fig. 5. Separation of pupae by the addition of water to the bowl; small (mostly male) pupae pass through the grid while large (mostly female) pupae are retained.

Fig. 6. Collection of separated pupae on nylon net at overflow spout from bowl.

Table 1. Separation of male and female *Culex pipiens fatigans* pupae using a grid with a 1.02-mm opening

Lot no. [a]	Emergence from small pupae [b]				Emergence from large pupae				Males		Females		Total no.: both sexes
	No. of males	No. of females	Total	Average percentage emergence [c]	No. of males	No. of females	Total	Average percentage emergence [c]	Total no.	% misclassified	Total no.	% misclassified	
1	354	9	363	97.5	8	199	207	94.0	362	2.2	208	4.3	570
2	223	8	231	92.0	9	97	106	97.0	232	3.9	105	7.6	337
3	152	4	156	96.0	0	118	118	97.0	152	—	122	3.3	274
4	164	20	184	97.0	4	178	182	96.0	168	2.4	198	10.1	366
5	166	14	180	92.0	7	101	108	96.0	173	4.0	115	12.2	288
6	1 323	38	1 361	97.0	19	1 021	1 040	98.0	1 342	1.4	1 059	3.6	2 401
total	2 382	93	2 475	95.3	47	1 714	1 761	96.3	2 429	1.9	1 807	5.1	4 236

[a] The different lots of pupae were from different pans of larvae reared under standard conditions at different times.
[b] Small pupae were those that passed through the grid.
[c] The average percentage that emerged from control lots was 96.4.

Results

The results of the separation of male and female *Culex pipiens fatigans* pupae with a grid with an opening of 1.02 mm are given in Table 1. The small pupae (those that passed through the opening of the grid) were a mixture of 97% males and 3% females, whereas those trapped beneath were 2.66% males and 97.34% females. There was no significant pupal mortality. Table 2 gives the results with the same grid over several days of pupation. The small pupae were 95% males and 5% females and the large pupae were 97% females and 3% males. In other words, 97% of the total number of males were collected with the small pupae, mixed with 5% of the total number of females.

Grids with smaller openings decreased the proportion of females collected, but also reduced the percentage of the total number of males collected. As shown in Table 3, a grid with openings of 0.92 mm reduced the proportion of females among the small pupae to only 1% but

Table 2. Separation of male and female pupae of *Culex pipiens fatigans* on consecutive days of pupation using a grid with a 1.02-mm opening

Day	Emergence from small pupae [a]			Emergence from large pupae			Males		Females		Total no.: both sexes
	No. of males	No. of females	Total	No. of males	No. of females	Total	Total no.	% misclassified	Total no.	% misclassified	
1	667	41	708	4	394	398	671	0.6	435	9.4	1 106
2	916	79	995	27	762	789	943	2.9	841	9.4	1 784
3	1 014	45	1 059	16	1 049	1 065	1 030	1.6	1 094	4.1	2 124
4	1 263	47	1 130	39	1 550	1 589	1 302	3.0	1 597	2.9	2 899
5	632	29	661	54	1 157	1 211	686	7.9	1 186	2.4	1 872
total	4 492	241	4 733	140	4 912	5 045	4 632	3.0	5 153	4.7	9 785

[a] Small pupae were those which passed through the grid.

Table 3. Separation of male and female pupae of *Culex pipiens fatigans* by grid with openings of 0.92 mm and 0.89 mm

Lot no.	Emergence from small pupae [a]			Emergence from large pupae			Males		Females		Total number: both sexes
	No. of males	No. of females	Total	No. of males	No. of females	Total	Total no.	% misclassified	Total no.	% misclassified	
					Grid with 0.92-mm openings						
1	625	7	632	44	811	855	669	6.6	818	0.9	1 487
2	485	4	489	68	1 065	1 333	553	12.3	1 069	0.4	1 622
total	1 110	11	1 121	112	1 876	1 988	1 222	9.2	1 887	0.6	3 109
					Grid with 0.89-mm openings						
1	268	0	268	793	1 536	2 319	1 051	74.5	1 536	—	2 587
2	106	0	106	320	499	819	426	75.1	499	—	925
3	344	0	344	386	1 755	2 141	730	52.9	1 755	—	2 485
total	718	0	718	1 489	3 790	5 279	2 207	67.5	3 790	—	5 997

[a] Small pupae were those which passed through the respective grids.

only 91% of the total number of males were collected, whereas openings of 0.89 mm eliminated all the females but yielded only 33% of the males. The former size could be used in the separation of males for sterilization and release, while the latter might be required in control experiments utilizing the cytoplasmic incompatibility technique or in genetic studies in which complete elimination of females is required.

Accuracy and speed in the separation of sexes in mosquitos is very important in mass releases in field experiments. While the available methods of sexing in the pupal stage are satisfactory for small experiments, they are not completely satisfactory for mass releases. The method discussed here is adaptable to mass-scale separations as well as laboratory use. It gives great accuracy and speed in sexing at the pupal stage depending upon the grid used.

In mass production of mosquitos for release a certain proportion of males must be returned to maintain the colony. Therefore any system of separation that harvests 90% or a higher percentage of the males and less than 5% of the females would be adaptable to sterile male release programmes.

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