

offer an excellent opportunity for further cytological studies on both mitosis and meiosis, as well as genetic studies on position effect when marker genes are available.

Furthermore, these translocation strains may find a use as starting materials in synthesizing new karyotypes.

SUMMARY

Six reciprocal translocations are described along with their methods of induction and maintenance. The possibility of the practical use of one of the translocations $T(Y;2R) 1$ in a future genetic control program is considered. Structural aberrations reported in this paper are promising for further studies of linkage group-chromosome correlations, position effects and building artificial karyotypes.

ACKNOWLEDGEMENTS

This study was supported in part by Grant AI09944 from the USPHS. The authors wish to thank Prof. H. S. Ducoff of the Department of Physiology and Biophysics, University of Illinois for allowing them to use his X-ray facilities, and Sharad Sethi of this department for help in isolating translocations.

References

- Curtis, C. F. 1968. A possible genetic method for the control of insect pests with special reference to tsetse flies. *Bull. Entom. Res.* 57:509-523.
- French, W. L., Baker, R. H. and Kitzmiller, J. B. 1962. Preparation of mosquito chromosomes. *Mosq. News* 22:377-383.
- Hobbs, J. H. 1962. Cytogenetics of *Anopheles albimanus*. *Ann. Entom. Soc. Amer.* 55:245-251.
- Keppler, W. J., Kitzmiller, J. B. and Rabbani, M. G. 1972. The salivary gland chromosomes of *Anopheles albimanus*. *Mosq. News* (in press).
- Laven, H. 1969. Eradicating mosquitoes using translocations. *Nature* 221:958-959.
- McDonald, P. T. and Rai, K. S. 1970. Correlation of linkage groups with chromosomes in the mosquito, *Aedes aegypti*. *Genetics* 66:475-485.
- Rai, K. S. 1967. Manipulation of cytogenetic mechanisms for genetic control of vectors. WHO publication SC/VG. 67.34, 12 pp.
- Rai, K. S. and Asman, Sr. M. 1968. Possible application of a reciprocal translocation for genetic control of the mosquito *Aedes aegypti*. *Proc. 12th Intern. Congr. Genet.* 1:164.
- Sakai, R. K., Baker, R. H. and Mian, A. 1971. Linkage group-chromosome correlation in a mosquito: translocations in *Culex tritaeniorhynchus*. *Jour. Heredity* 62:90-100.
- Serebrovskii, A. S. 1940. On the possibility of a new method for the control of insect pests. *Zool. Zhurnal* 19:618-630. (in Russian, translated by C. F. Curtis).
- Wagoner, D. E. 1967. Linkage group-karyotype correlation in the house fly determined by cytological analysis of X-ray induced translocations. *Genetics* 57:729-739.
- Wagoner, D. E., Nickel, C. A. and Johnson, O. A. 1969. Chromosomal translocation heterozygotes in the house fly. *Jour. Heredity* 60:301-304.

LABORATORY AND FIELD EVALUATION OF A SONIC SIFTER AS A MOSQUITO EGG EXTRACTOR

TAKESHI MIURA

University of California, Mosquito Control Research Laboratory, Fresno, California 93727

ABSTRACT. A sonic sifter proved to be a simple and dependable device for extracting aedine mosquito eggs from air-dry soil samples. It gave a high recovery rate (91.68 percent) of samples seeded with a known number of *Aedes nigromaculis* (Ludlow) eggs. There was no effect on

hatchability of the eggs which had been sifted for 1 to 10 minutes. Samples collected from dense vegetated areas contained the most eggs; samples collected from the bottom of a depression with no vegetation contained the least eggs.

The eggs of aedine mosquitoes are usually laid in moist places near larval habitats. In the Central Valley of California, *Aedes nigromaculis* (Ludlow) breed in large numbers in irrigated pastures; however, little information is available on their oviposition behavior. Husbands and Rosay (1952) reported that eggs of this species were found in damp places in irrigated pastures.

Very little attention has been paid to destruction of mosquito eggs either by the direct application of chemicals to the oviposition sites or by the indirect method, such as irrigation practices (Lopp, 1957). Usually to study oviposition sites in nature, soil samples collected from suspected areas are examined by a mechanical means (Gjullin, 1938; Husbands, 1952; Horsfall, 1956; Service, 1968) or indirectly by hatching the eggs by submerging the soil samples (Filsinger, 1941; Bodman and Gannon, 1950). A majority of eggs of *A. nigromaculis* hatch readily in the first flooding (Husbands, 1952); however, some of the eggs have shown a delayed hatch, especially those laid in late season (Miura *et al.*, 1968). Therefore, both the

mechanical and flooding methods which require water (Horsfall, 1956; Service, 1968) are not reliable to examine population densities of the eggs. To overcome these problems, a sonic sifter originally designed for particle-size analysis (Figure 1) was used to extract *A. nigromaculis* eggs from air-dry soil samples.

This paper reports an evaluation of the sonic sifter as a means of separating mosquito eggs from soil samples.

MATERIALS AND METHODS. To determine the correct sieve mesh for retaining eggs, measurements were made on 100 *A. nigromaculis* eggs randomly collected from a colony which has been maintained by induced mating (Miura, 1969). Length and width were measured under a stereoscopic microscope at a magnification of 27 diameters.

To evaluate the efficiency of egg separation by the sonic sifter (Allen-Bradley, Model L3P), 30 soil samples, each weighing about 20 g, were taken from a field which had never been used as an irrigated pasture in recent years and these samples were seeded with eggs. Number, mesh of sieves, amplitude, and pulse of sift are

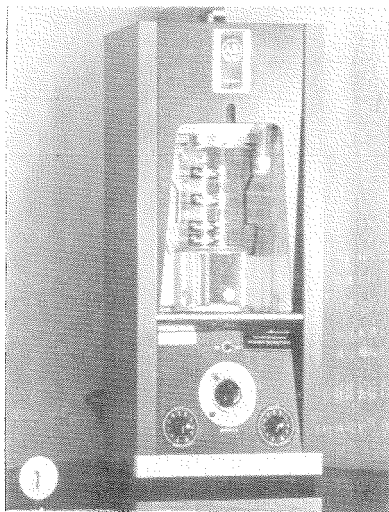


FIG. 1.—The sonic sifter used for mosquito egg extraction from soil samples.

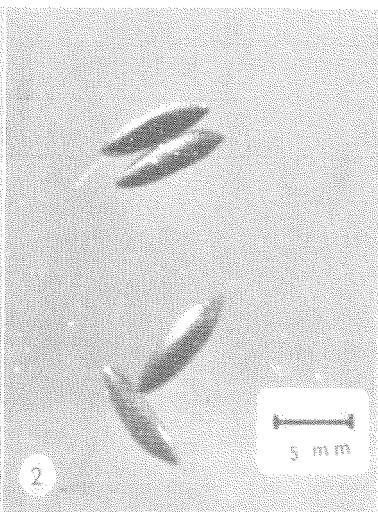


FIG. 2.—Eggs of *Aedes nigromaculis*.

largely dependent upon the soil types; however, five sieves (14-, 40-, 60-, 80- and 100-mesh) were used with the average sandy loam soil. Amplitude and pulse controls were set at "5" and "4," respectively, and each sample was then sifted for 5 minutes. The material in the 80- and 100-mesh sieves and fines collector was carefully examined with a stereoscopic microscope for eggs.

To check the effect of the efficiency of egg recovery by different people, two assistants were assigned the task of recovering eggs and a record was kept of the number of eggs recovered and the time required to examine each sample.

To evaluate the efficacy of using the sifter for extracting eggs from soil samples taken from oviposition sites, a small enclosed plot (10 x 10 x 3 dm) was established consisting of a 15 cm layer of top soil enclosed by plywood board lined with polyethylene sheeting and provided with a removable screen top; a mixture of grass seeds (ryegrass, fescue, Kentucky blue grass, and bent grass) was added; the vegetation was well established within a month and maintained easily. The tests were conducted using field-collected *A.*

nigromaculis adults; approximately 200 gravid females were placed on the enclosed plot and held there for 5 to 6 days.

Samples measuring 25 x 25 mm were cut to a depth of 10 mm from five likely oviposition sites in the plot and the samples were then sifted and examined for eggs as described previously.

Description of five sampling sites in the plot are as follows:

- A. At the bottom of a depression with no vegetation.
- B. At the open bare soil among grasses.
- C. At the base soil under grass leaves.
- D. At the base of grasses.
- E. At the edge of the plot.

RESULTS AND DISCUSSION. *A. nigromaculis* eggs are black and elongate-oval-shaped with the anterior end more rounded than the posterior (Figure 2). The mean length was 0.664 ± 0.004 mm, range 0.600 to 0.767; the mean width was 0.182 ± 0.001 mm, range 0.167 to 0.200. Most of the eggs were retained by an 80-mesh sieve (openings 0.177 mm); however, about 10 percent of the eggs passed through that sieve but were retained by a 100-mesh sieve (openings 0.149 mm). There were

TABLE 1.—The efficiency of the sonic sifter in extracting mosquito eggs from air-dry soil samples.

Replication	Lab assistant A			Lab assistant B		
	Eggs seeded	% Recovered	Time (min)	Eggs seeded	% Recovered	Time (min)
1	19	94.74	16	100.00
2	19	100.00	17	100.00
3	20	95.00	18	88.89
4	18	94.44	19	84.21
5	15	86.67	100	14	68.75	165
6	17	94.12	95	17	58.82	165
7	20	90.00	105	19	94.74	165
8	12	100.00	60	15	86.67	95
9	13	92.31	110	14	100.00	100
10	15	86.67	95	11	90.91	87
11	17	88.24	90	12	100.00	90
12	18	100.00	115	16	93.75	80
13	15	86.67	90	15	100.00	110
14	15	93.33	105	14	100.00	110
15	16	87.50	110	16	93.75	75
Mean		92.46	97.7		90.70	112.9
S.E.		1.31			3.17	

TABLE 2.—Oviposition preference of *A. nigromaculis* for five categories of sampling sites in an experimental plot.

Category	Replication					Mean ^a
	1	2	3	4	5	
A. The bottom of depression	4	2	4	3	0	2.6 (1.7)
B. Bare soil	2	0	7	3	4	3.2 (1.8)
C. Under grass leaves	7	10	19	8	0	8.8 (2.8)
D. Base of grass	29	29	82	5	18	35.8 (5.3)
E. Edge of the plot	1	3	54	20	15	18.6 (3.8)

^a Number in parentheses is calculated from the square root transformed number ($\sqrt{Xi + 0.5}$) of the original data.

many fragments of eggshells in the fines collector, but no entire eggs were found.

The percentage of the eggs recovered from the 30 samples (482 eggs) varied from 58.82 percent to 100 percent. The mean recovery rate was 91.68 ± 1.34 percent. To check the differences in the efficiency of egg recovery by the two assistants, recovery rates were recorded for each sample by each assistant (Table 1). The mean recovery rate by Assistant A was 92.65 ± 1.31 percent (range 86.67 to 100) and by Assistant B was 90.70 ± 3.1 percent (range 58.82 to 100). Statistically, the difference in the means (1.95 percent) is not significant at the 0.01 level ($t=0.551$). However, it is interesting to point out that Assistant B recovered less eggs and obtained about 2.4 x larger standard error of the mean than those of Assistant A; furthermore, he required 15 minutes more time to examine a sample (Table 1).

Time required to check a sample will be

influenced by the amount of debris in the sample as well as the type of soil. With a fairly clean, sandy loam, an average of about 1 hour 45 minutes was required to examine a sample.

Effect of the sonic sifter on viability of eggs is probably nil. About 800 eggs have been sifted for 1 to 10 minutes without any detectable effect on their hatchability.

Although the females laid eggs in soil at all categories in the test plot (Table 2), they showed a pronounced preference for the sites offering much vegetation. In order to perform the analysis of variance test, the original data were transformed into the square root ($\sqrt{Xi + 0.5}$). The test is highly significant at the 5 percent level ($F=3.91$). About 52 percent of the eggs obtained were from the samples collected at the base of dense grass (Category D), 27 percent was from the edge of the plot (E), and 13 percent was from the bare soil under the grass leaves (C). Smaller numbers of eggs (4 percent) were collected from the bottom of a depression with no vegetation (A) and 5 percent from the samples at the bare open area (B).

To compare a mean of eggs laid at a category with those of other categories, the Student-Newman-Keul's multiple-range test was used. The result of the test is shown in Table 3. Category D has a significantly higher mean than those of other categories; however, the differences among means of Categories C, D, and E are not great enough to be significant at

TABLE 3.—The results of the Student-Newman-Keul's multiple-range test on the data obtained from the ovipositional preference of *A. nigromaculis* in an experimental plot.

Category	A	B	C	E	D
Mean	1.7	1.8	2.8	3.8	5.3

^a Any two means not underscored by the same lines are significantly different at the 5% level.

the 5 percent level. From Category A, the least number of eggs were obtained though the differences from B, C, and E are not significant.

References Cited

- Bodman, M. T. and Gannon, N. 1950. Some habitats of eggs of *Aedes vexans*. J. Econ. Entomol. 43:547-548.
- Filsinger, C. 1941. Distribution of *Aedes vexans* eggs. N. Jers. Mosq. Exterm. Assoc. Proc. 28:12-19.
- Gjullin, C. M. 1938. A machine for separating mosquito eggs from soil. U.S. Dept. Agric., Bur. Entomol. & Pl. Quar., Circ. ET 135, 14 pp.
- Horsfall, W. R. 1956. A method for making a survey of floodwater mosquitoes. Mosq. News 16:66-71.
- Husbands, R. C. 1952. Some techniques used

- in the study of *Aedes* eggs in irrigated pastures in California. Mosq. News 12:14-50.
- Husbands, R. C. and Rosay, B. 1952. A cooperative ecological study of mosquitoes of irrigated pastures. Calif. Mosq. Cont. Assoc. Proc. 20: 17-26.
- Lopp, O. V. 1957. Egg sampling as an index of mosquito breeding. N. Jers. Mosq. Exterm. Assoc. Proc. 44:60-63.
- Miura, T. 1969. Evaluation of techniques used for mass rearing *Aedes nigromaculis* by induced mating. Mosq. News 29:612-616.
- Miura, T., Husbands, R. C. and Wilder, W. H. 1968. Observations on the hatching of *Aedes nigromaculis* (Ludlow) eggs (Diptera:Culicidae.) Calif. Mosq. Cont. Assoc. Proc. 36: 42-43.
- Service, M. W. 1968. A method for extracting mosquito eggs from soil samples taken from oviposition sites. Ann. Trop. Med. Parasitol. 62:478-480.

PHORETIC ATTACHMENT OF *SIMULIUM* LARVAE AND PUPAE TO MAYFLY AND DRAGONFLY NYMPHS¹

GEORGE J. BURTON² AND THOMAS M. McRAE³

BACKGROUND. Phoresy among insects is a type of relationship in which an insect is carried on the body of another, larger insect, but does not feed on the latter (Torre-Bueno, 1962). Phoretic attachment of *Simulium* larvae and pupae to mayfly and dragonfly nymphs has been reported mostly in Africa. The attached form may be designated as the "epizoite," and its host a "carrier." (Clausen, 1962; Grenier

and Mouchet, 1958). Both terms "larvae" and "nymph" appear in the literature in reference to aquatic stages of Odonata and Ephemeroptera. Chapman (1969) states that the stage which succeeds the egg is a larva, and that the nymph is a larva of a hemimetabolous insect which basically resembles the adult, in contrast with the radically different larva of a holometabolous insect. Phoretic attachment of *Simulium* species in Africa occurs also upon crabs and prawns, but will not be reported here.

The nymphs of many species of mayflies and dragonflies inhabit fast-flowing water, and hence occur in large numbers in the same places where blackfly larvae develop. A blackfly larva which has sufficient food carried to it by the current in a favorable and undisturbed environment,

¹ This work was done while the authors were assigned to the National Institutes of Health (U.S.A.)—National Institute of Health and Medical Research (Ghana) Joint Research Program, Accra and Bolgatanga, Ghana.

² National Cancer Institute, Federal Building, Room 508, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.

³ Department of Entomology, University of Queensland, St. Lucia, Brisbane, Queensland, Australia 4067.