

# FEMALE-LETHAL, A HERITABLE FACTOR IN *CULEX PIPPIENS* L.

A. RALPH BARR<sup>1</sup> AND C. M. MYERS<sup>2,3</sup>

**ABSTRACT.** A heritable factor is described which results in the death of all, or practically all, females in a progeny. The typical "lethal" egg raft contains approximately equal numbers of viable and dead eggs. Larvae hatching from

such rafts give rise almost invariably to males. Experiments bearing on the inheritance of the factor are described but the mode of inheritance is not yet understood.

## INTRODUCTION

Possible approaches to the control of mosquitoes by genetic methods have been discussed at some length by an expert committee of the World Health Organization (World Hlth. Org., 1964). This committee emphasized the paucity of deleterious factors which had been described in mosquitoes and urged that the search for such factors be intensified. This is a report of a previously undescribed lethal factor the inheritance of which is not yet understood.

## MATERIALS AND METHODS

Female-lethal (*l<sup>t</sup>*) was first noticed after inbreeding adults of the Orange County (California) laboratory strain of *Culex pipiens quinquefasciatus* Say for a single generation. Several rafts were laid in which approximately one-half of the embryonated eggs failed to hatch. All adults reared from the larvae which did hatch were male. These males were mated with female cousins which in turn produced some rafts which gave rise exclusively to males. The line was maintained for about 24 generations by such cousin crosses but this degree of inbreeding resulted in an accumulation of deleterious factors of irregular inheritance. The strain was successfully outcrossed on two occa-

sions which resulted in a temporary increase in viability.

Adults were kept in small cages of various sizes. Chickens were used as a source of blood; the colony remained anautogenous even though some genetic material from an autogenous strain was introduced in one of the outcrosses.

Tap water was provided for oviposition. Egg rafts were hatched in paper cups (coated with wax) one raft to a cup; each raft was freed from all loose eggs which might have come from other rafts. After an interval of time sufficient for hatching, usually two or three days after placement in the cup, the hatched rafts were removed for examination.

Rafts in which the lethal was expressed could usually be detected by the paucity of hatched larvae. In addition, hatching tended to occur over a longer period of time than normal, there frequently being much variation in the size of hatched larvae. It was common for substantial numbers of larvae to die while hatching or soon thereafter; this was especially likely when fewer than one-half of the embryos died. Lethal rafts also tended to disintegrate after hatching to a lesser extent than did normal ones.

An attempt was made to rear at least four lethal progenies in each generation. After hatching was complete the larvae were counted. The hatched raft was removed from its cup with a fine brush and placed on moist filter paper. It was teased apart with a dissecting needle while being viewed at a magnification of 10 or 16 diameters. Unhatched eggs were scored as "undeveloped" or "dead embryo" depending on whether or not embryonic

<sup>1</sup> School of Public Health, University of California, Los Angeles, 90024.

<sup>2</sup> Bureau of Vector Control and Solid Waste Management, California Department of Public Health, Fresno, 93721.

<sup>3</sup> This research was supported by Public Health Service research grant CC 00367, from the Center for Disease Control, Atlanta, Georgia.

development could be detected. Undeveloped eggs presumably were not fertilized; a few such eggs are usually found in otherwise normal rafts. Occasional eggs were obviously abnormal (misshapen, failed to darken, etc.); these were never common and almost never evidenced embryonic development.

Larvae were reared in tap water which was aerated; they were fed an alfalfa-base, livestock supplement. Temperature, humidity, and light were not carefully controlled. Pupae were sexed by the terminalia as described by Barr (1954).

## RESULTS

CHARACTERIZATION OF LETHAL. An analysis of several lethal rafts is shown in Table

1 was female. The lethal raft is thus made up of roughly equal numbers of viable and dead eggs, and the viable eggs produce almost exclusively males. It would appear that practically all females die during embryonic development.

It must be re-emphasized that the figures in Table 1 are drawn only from rafts in which 90 percent or more of the larvae survived to the pupal stage. In Table 2 are shown data from some rafts in which the number of dead eggs was much smaller than the number of viable eggs. Larval survival in these rafts ranged from only 66 to 79 percent. If it is assumed, however, that one-half of the fertile eggs were male, the expected number of males was very close to the number actually

TABLE 1.—Typical data from lethal egg rafts.

No.	Total eggs	No. undeveloped	Total fertile	Dead embryos	Larvae	Pupae		
						Male	Female	Total
1	261	110	151	73	78	70	0	70
2	258	0	258	95	163	162	0	162
3	231	7	224	114	110	108	0	108
.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.
35	192	10	182	91	91	83	0	83
Total	7364	469	6895	3286	3609	3388	28	3416
Mean	210.4	13.4	197.0	93.9	103.1	96.8	0.8	97.6*

\* 95% survival.

1. These data are from all rafts that were reared in which there was a large number of eggs with dead embryos and in which 90 percent or more of the larvae which hatched survived. Several times this many lethal rafts were reared but in most cases larval survival was poor. The figures in Table 1 show that on the average the lethal rafts contained 210 eggs of which 13 showed no development and were presumably infertile. Infertile eggs, however, tended to occur more commonly in some rafts than in others; the median number was 5 and the mode 3.

Of the average of 197 eggs which evidenced development, 94 died and 103 hatched. Of these 103 larvae, 98 survived to pupation of which 97 were male and

reared. In these rafts it would appear that many female larvae did not die as embryos, as they normally do, but were able to hatch successfully. In rafts of this type it was common to see many dead first-stage larvae, sometimes on the bottom of the container, sometimes protruding from the eggs.

Well over 100 egg rafts were reared in which there were roughly equal numbers of dead and viable eggs. Of these only one or two gave rise to substantial numbers of females. All of the rest produced only males or an occasional female.

Fairly large numbers of rafts were seen in which there were substantially fewer dead eggs than viable ones. Occasionally one of these proved to be a lethal raft

TABLE 2.—Lethal rafts in which there were too few dead eggs.

Raft	Total eggs	No. undeveloped	Total fertile	Dead embryos	Larvae	Pupae			Expected *
						Male	Female	Total	
1	257	1	256	52	204	134	0	134	128
2	136	1	135	44	91	65	0	65	68
3	293	0	293	101	192	147	0	147	147
4	285	0	285	110	175	139	0	139	143
5	219	2	217	65	152	105	0	105	109

\* One-half of "total fertile" (column 4).

(as in Table 2) but most produced progenies with normal sex ratios.

After prolonged inbreeding of the line it was common to see rafts in which there were many more dead than viable eggs. Such rafts produced either "male-only" or normal progenies. The dead eggs in progenies which had a normal sex ratio were probably due to other types of egg lethals of irregular inheritance which accumulate with inbreeding but which are not manifested in outcrosses.

**SEX RATIO IN LETHAL PROGENIES.** From the 35 high-survival rafts utilized for Table 1, 28 females were reared; this was less than 1 per raft of all adults reared from the rafts. Twenty-two of the rafts produced only males; of the remaining 13 one produced 7, one 6, and the other 11 produced only one or two females each. In a much longer series, which included low survival rafts, 117 of 152 rafts gave rise to males only; the other 35 rafts produced 1 to 7 females, 22 of the 35 produced only one female each.

The origin of these occasional females is not certain. It is possible that at least some were due to contamination. Genetic markers were used to ascertain whether or not the females were contaminants. In one case contamination seemed probable but in several other cases the females carried markers which made contamination an unlikely explanation. In three instances such female survivors were inbred with sibling ("male-only") males and in each case they laid rafts which hatched normally.

**INCIDENCE OF LETHAL RAFTS IN COUSIN CROSSES.** Males from "male-only" rafts were routinely bred with female cousins. This

was done for 24 generations during which time about 1400 rafts were examined, of which 18.6 percent exhibited the lethal. In different generations the proportions exhibiting the lethal ranged from 0 to 34 percent. It would appear that the large variations in incidence from one generation to another are not chance variations but reflect generations more or less prone to produce lethal rafts, for reasons not understood.

In one generation all female cousins were randomized between two cages. "Male-only" male cousins were added to one of the cages and normal sibling males to the other. In this case 23 of 79 (29 percent) rafts from the lethal-male cage were lethal as were 14 of 57 (25 percent) from the normal-male cage. These rather surprising results indicated that females of the lethal strain produced lethal rafts as readily when mated with siblings as when mated with males from lethal rafts.

A similar experiment was done in the following generation with similar results; 10 of 37 females laid lethal rafts when mated with males from lethal rafts while 14 of 63 laid lethal rafts when mated with sibling males.

**OUTCROSSING OF "FEMALE-LETHAL."** The "female-lethal" characteristic was on two occasions outcrossed and recovered. In both cases males from lethal rafts were crossed with females of independent strains and some lethal rafts were laid by  $F_2$  or  $F_3$  females. In the first case several males from a male-only raft were crossed with females of a yellow-larva ( $y$ ) strain. A single  $F_1$  raft (progeny 4 in Table 3) was reared and the adults inbred. The females

TABLE 3.—Progenies which produced lethal rafts when inbred.

Progeny	Total eggs	Undev.	Total Fertile	Embryos	Larvae	Pupae		
						Male	Female	Total
1	95	0	95	1	94	14	25	39
2	109	0	109	0	109	49	50	99
3	116	0	116	0	116	69	37	107
4	160	1	159	4	155	72	77	149

after feeding produced six rafts of which two were lethal.

On a second occasion males from a male-only raft were crossed with females of a yellow-larva, ruby-eye (*y ru*) strain (Iltis *et al.*, 1965). Four normal  $F_1$  rafts were reared and the adults inbred. Four  $F_2$  rafts were examined and all were normal. The  $F_2$  adults were inbred and in the  $F_3$  there were 5 lethal and 36 normal rafts.

On two other occasions the strain was outcrossed but the lethal was not recovered. In one case males from male-only rafts were outcrossed to white-eye (*w*) females (Gilchrist and Haldane, 1947). Upon inbreeding, a previously unrecognized sex-linked lethal from the white-eye strain was found. This lethal so interfered with the study that the original lethal could not be recovered.

On a second occasion males from male-only rafts were crossed with red-eye (*r*) females (Wild, 1963). The lethal was not recovered even though large numbers of egg rafts were examined during each of six generations of inbreeding. It is perhaps significant that  $h^+$  was recovered on the two occasions when it was outcrossed to strains bearing markers for the second chromosome (*y, ru*) but was not recovered on either occasion that it was outcrossed to strains bearing markers for the sex chromosome (*w, r*).

SEX RATIOS OF PARENTS OF LETHAL EGG RAFTS. On four occasions lethal rafts have been laid after inbreeding of apparently normal progenies (Table 3). Rafts, 1, 2 and 3 represent the original discovery of the lethal. In none of these rafts were there many infertile or dead eggs. The true sex ratio of progeny 1 is questionable because so few of the larvae survived. In the other three progenies, however, there were significantly more males than females in progeny 3 ( $p < .01$ ) but not in progenies 2 or 4. In each of the first 3 progenies, one male was mated with his female siblings. The incidence of lethal rafts in the  $F_2$  was 5 of 5 from progeny 1, and 2 of 4 in both progenies 2 and 3. All adults of progeny 4 were inbred; 2 of 6 rafts produced were lethal.

In the normal course of maintaining the lethal, males from a lethal raft were crossed with female cousins. It was customary to use females from many rafts to lessen the effect of inbreeding. In a few cases, however, males were crossed with females from a single raft. The data in Table 4 do not indicate different kinds of females within a progeny, although only 4 females were tested. In raft 1 there were significantly more males than females; this male excess, however, was associated with high larval mortality which could have fallen mostly on females. In the other three

TABLE 4.—Egg rafts produced by female siblings when mated with male cousins from a lethal raft.

Raft	Total eggs	Undev.	Total Fertile	Embryos	Larvae	Pupae		
						Male	Female	Total
1	287	1	286	3	283	139	92	231
2	209	15	194	7	187	82	77	159
3-4	508	0	508	1	507	258	219	477

rafts there were not significantly more males than females.

INHERITANCE OF "FEMALE-LETHAL." Individuals were prepared which were heterozygotic for two autosomal markers, *y* and *ru* (Iltis *et al.* 1965), in coupling phase; these are markers for linkage-group II. Males from lethal rafts were crossed with female cousins; double heterozygotes were crossed back to double homozygotes in both directions. The results (Table 5)

In Table 6 are shown three types of back-crosses of *de*, one with female heterozygotes and the other two with male heterozygotes (one with maleness in coupling with *de*, the other with maleness in repulsion with *de*). The lack of penetrance of *de* is immediately evident from the data. In spite of this, however, it would appear that sex determination is normal in "male-only" rafts.

TABLE 5.—Backcrosses of males from male-only rafts (two autosomal markers in coupling).

Heterozygotic parent	No. progenies	Offspring								Total
		Male				Female				
		<i>y</i>		+		<i>y</i>		+		
		<i>ru</i>	+	<i>ru</i>	+	<i>ru</i>	+	<i>ru</i>	+	
Male	3	54	11	6	65	0	0	0	1	137
Female	11	362	70	77	311	2	1	2	10	835
Total	14	416	81	83	376	2	1	2	11	972

indicated no irregularity in distribution of chromosome II in the lethal cross.

A previously unknown mutant, divided-eye (*de*) appeared spontaneously in the lethal strain (Barr, 1969). The factor proved to be incompletely penetrant (with a higher degree of penetrance in females), variably expressed, sex-linked, and recessive. Studies not detailed here indicate that *de* is about 30 crossover units from the factor controlling sex and on the opposite side of the sex factor from *r* and *w*.

## DISCUSSION

When it is observed that half of the eggs in a raft die while the other half produce only males, it seems likely that the half which died were genetically intended females. This assumption agrees with all of the evidence thus far. In the third line of Table 6, for example, *de* and + male offspring occurred in roughly equal numbers, which indicates an approximately equal contribution of *de* and + gametes

TABLE 6.—Backcrosses of *de* in lethal progenies.

Parents			Offspring				Total	
Male	Female	Progenies	o		o			
			<i>de</i>	+	<i>de</i>	+		
M <i>de</i> m +	m <i>de</i> m <i>de</i>	13	44	1	202	1	2	646
M + m <i>de</i>	m <i>de</i> m <i>de</i>	5	63	233	0	1		297
M <i>de</i> m <i>de</i>	m + m <i>de</i>	14	320	399	0	3		722

from the female parent. Such data make improbable the hypothesis that for some reason all of the offspring were male and only coincidentally half of them died.

When the lethal was first discovered it was found that affected rafts had many eggs which did not hatch in the normal manner, by a circular rupture which delineates the operculum. These abnormal eggs seemed to have merely exploded; there appeared to be no operculum at all. It was also noticed that larvae hatched over a period of 3 days or so rather than the normal 2 days. Many of the embryos remained alive but unhatched for a day or so after the other larvae had hatched. It was therefore thought that female eggs were improperly formed. This does not seem to be an adequate explanation of the lethal since female larvae appear not to survive even when they hatch successfully (as in Table 2), female embryos do not survive well even when mechanically removed from the egg, the "exploding" egg has not been seen constantly in this strain, and it has been seen in other strains in which it did not appear to be associated with lethality.

The inheritance of "female-lethal" is not yet understood. If it were a simple dominant it could not be inherited at all since any individual carrying it would die in the egg stage. If it were a dominant expressed only in females it would be expressed when outcrossed, which was not the case. If it were a recessive it could kill no more than one-half of the female offspring since a female could transmit the factor to no more than one-half of her daughters; this is true regardless of whether or not the lethal affected males. No satisfactory model has yet been devised to account for the findings.

## CONCLUSIONS

1. A factor ( $l^v$ ) is described which results in the death of practically all females before they hatch from eggs. Such "lethal rafts" usually contain approximately equal numbers of dead and viable eggs.

2. In this strain rafts with markedly more dead than viable eggs may or may

not give rise to females. Such an excess of dead embryos apparently is caused by other lethals which may or may not be superimposed on  $l^v$ .

3. Rafts with many dead embryos but with substantially more viable than dead eggs may or may not give rise to females. "Lethal rafts" with too few dead embryos usually show large numbers of dead first-stage larvae.

4. Occasional females are produced from lethal rafts. In some cases such females have carried markers which indicated that they were probably not contaminants. Several such females have been bred with male siblings but none produced a lethal raft. The numbers, however, were small.

5. When "male only" males are bred to female cousins about 19 percent of the progenies produced are lethal; the variability in this figure, however, suggests that there are real differences in incidence in different generations. In two experiments such females produced similar numbers of lethals when mated with male siblings.

6. The factor has been transmitted to clean strains by outcrossing males. Lethal rafts were produced after inbreeding for one or two generations.

7. Females which produce lethal rafts may or may not have come from rafts in which there was a normal sex ratio.

8. A male produces lethal rafts when mated with some but not all female siblings.

9. Backcrosses of individuals heterozygotic for markers on the first and second chromosomes indicate no irregularity of distribution of these chromosomes in lethal rafts.

10. A satisfactory hypothesis for the inheritance of  $l^v$  has not yet been devised.

## References

- Barr, A. R. 1954. Hybridization experiments with some American dark-winged anophelines. *Exper. Parasitol.*, 3:445-57.
- Barr, A. R. 1969. Divided-eye, a sex-linked mutation in *Culex pipiens* L. *J. Med. Entomol.*, 6:393-7.
- Gilchrist, B. M. and J. B. S. Haldane. 1947. Sex linkage and sex determination in a mosquito, *Culex molestus*. *Hereditas*, 33:175-90.
- Iltis, W. G., A. R. Barr, G. A. H. McClelland and C. M. Myers. 1965. The inheritance of

yellow-larva and ruby-eye in *Culex pipiens*. Bull. World Hlth Org., 33:123-8.  
 Wild, A. 1963. A red eye colour mutation in *Culex pipiens* after X-irradiation. Nature, 200: 917-8.

World Health Organization. 1964. Genetics of Vectors and Insecticide Resistance. Report of a WHO scientific group. Geneva: WHO Tech. Rep. Ser., No. 268, 40 pp.

## TRANSOVARIAN TRANSMISSION OF BLUETONGUE VIRUS UNLIKELY FOR *CULICOIDES VARIIPENNIS*

ROBERT HENRY JONES<sup>1</sup> AND N. M. FOSTER<sup>1</sup>

**ABSTRACT.** Transovarian transmission of bluetongue virus was not demonstrated for colony *Culicoides variipennis* (Coquillett) (Diptera: Ceratopogonidae) when the first to the fifth egg

batches of infected females were reared and progeny females were given a normal blood meal, incubated, and then assayed for the presence of virus.

The ability of a virus to persist in an area after an epizootic is important to the epidemiology of a viral disease. The possibility of persistence of the virus would be increased by transovarian transmission in the vector, that is, if the infective agent passes through the egg to the next generation. If activity of the vector were seasonal, transovarian transmission would allow the virus to overwinter in a sheltered, immature form of the insect, such as the overwintering larval form of *Culicoides variipennis* (Coquillett) in Colorado (Jones, 1967a).

Burgdorfer and Varma stated in their review (1967) that, although transovarial development of arboviruses takes place in ticks, there is no conclusive evidence for this in mosquitoes and the evidence for it in phlebotomines has not been settled. Because transovarian transmission of arboviruses has not been conclusively demonstrated for any species of biting fly, we have not considered it likely for *C. variipennis*, the probable primary vector of bluetongue (BT) disease of sheep and cattle in the United States, and we have not

found it expedient to test for it until recently.

Because the flies used in our research program with the transmission of BT virus needed further standardization, we developed insect rearing facilities to conduct genetic studies with infected flies. A desirable first step was to determine the feasibility of dividing the parent colony of *C. variipennis*, established in 1957 and maintained without the addition of wild flies (Jones *et al*, 1969), through genetic selection into divergent lines resistant and susceptible to the transmission of BT virus. The selection process involved the individual mating and subsequent rearing of progeny of single females that were killed and assayed for virus after they had deposited one or more egg batches. Because normally a second, and occasionally a third, egg batch was also collected, the possibility of the infection of some progeny by transovarian transmission was greater than if we had used only the first egg batch. This statement assumes that the reproductive system, or some part of it, might be resistant to infection, or at least not become infected within the first few days needed to deposit a first egg batch after ingestion of the infective blood meal.

Clearly, we had to show that the positive assay results in our genetic program were not caused by transovarian transmis-

<sup>1</sup> R. H. Jones is of the Entomology Research Division and N. M. Foster of the Veterinary Sciences Research Division, Agr. Res. Serv., U. S. Department of Agriculture, Denver, Colorado 80225.