GENE-CONTROLLED MORPHOLOGICAL DIFFERENCES IN MALE GENITALIA OF *AEDES AEGYPTI* AND *AEDES MASCARENSIS* (DIPTERA: CULICIDAE)[1]

W. K. HARTBERG[2] AND G. B. CRAIG, JR.
Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556

ABSTRACT. Crossing experiments indicate that a single gene, Terminalia (*T*), controls shape of telomeres and large hairs on the basal lobes of male genitalia of *Aedes aegypti* and *A. mascarensis.* The gene is sex-linked and shows incomplete dominance. Phenotypes include genitalia like *A. aegypti* (*TT*), resembling *A. mascarensis* (T^mT^m), and intermediate as in the F_1 hybrid (TT^m).

Aedes aegypti is a tropicopolitan species, found associated with man throughout the warmer regions of the world. *Aedes mascarensis* is a species with very restricted distribution. It is limited to the island of Mauritius in the Indian Ocean. It is closely related to *A. aegypti;* both are members of subgenus *Stegomyia*, Group A.

Mattingly and Bruce-Chwatt (1954) considered *A. mascarensis* to be the closest relative of *A. aegypti.* Later, Mattingly (1966) stated that *A. mascarensis* should be considered a subspecies of *A. aegypti.* He based this classification on the successful hybridization and production of fertile offspring reported by McClelland and Mamet (1962). Hartberg and Craig (1968, 1970) have demonstrated positive sexual isolation and hybrid breakdown between *A. mascarensis* and *A. aegypti.* When the data from these studies are combined with the excellent discussion of the ecological and historical relationships between *A. aegypti* and *A. mascarensis* by McClelland (1967), the evidence is sufficient for reconsidering these two mosquitoes as separate species. The two species show numerous morphological and behavioral differences (McClelland, 1962). Among these are several morphological differences in the male genitalia of the two species.

Several papers have been published describing the male terminalia of *A. aegypti.* Among the most recent are papers by Hodapp and Jones (1961) and Spielman (1964). The male genitalia of *A. mascarensis* have only been briefly mentioned by Edwards (1941).

Since there seem to be no barriers to hybridization, (McClelland and Mamet, 1962), it is possible to use mutants from *A. aegypti* in the genetic analysis of *A. mascarensis* or vice versa. In the present work a marked strain of *A. aegypti* was crossed to *A. mascarensis* in order to study the genetic basis of the genitalic characters of the two species.

Materials and Methods. The mosquito strains used in this work were obtained from laboratory colonies maintained at the Vector Biology Laboratory, University of Notre Dame, Notre Dame, Indiana (Table 1). Strains used for most of the work were RED EYE of *A. aegypti* and MASC-O of *A. mascarensis.* The other strains were used for comparative purposes.

Rearing methods for obtaining reasonably standardized mosquitoes are given by Craig and VandeHey (1962) and these methods were generally used in the present work.

All crosses were made with virgin mosquitoes. F_1 hybrids were obtained from RED EYE females x MASC-O males and MASC-O females x RED EYE males. These F_1's were then backcrossed to the parental types. All eight possible backcrosses were made.

The following procedure was used to

[1] This work was supported by National Institutes of Health Research Grant No. AI-02753.

[2] Present Address: Department of Biology, Institute of Arthropodology and Parasitology, Georgia Southern College, Statesboro, Ga. 30458.

Table 1.—Strains of *Aedes* used in this investigation.

Species and form	Strain	Strain composition
A. aegypti aegypti	RED	Multiple marker strain synthesized in the laboratory. Contains chromosome markers—I: sex, red eye; II: spot abdomen, yellow larva; III: black-tarsi.
	BLPCO	Homozygous for mutants black-palp and compressed antenna on chromosome III. Inbred by single-pair, brother-sister matings to F_9.
	MINBLT	Homozygous for mutants miniature appendages (*min*) and black-tarsi (*blt*).
	WART	Homozygous for wart palp (*wa*) on chromosome II.
	NIH	A laboratory strain from the National Institutes of Health; maintained in the laboratory at least 38 years. Wild-type, no marker genes.
	ROCK	A laboratory strain from the Rockefeller Institute; maintained in the laboratory at least 30 years. Wild-type, no marker genes.
A. aegypti formosus	SSISA	Collected from eggs deposited in bamboo pots, Ssisa, Uganda, by J. D. Gillett, East African Virus Research Institute, 1960. Adults black and silver, active movement.
A. mascarensis	MASC-O	Laboratory colony of G. A. H. McClelland, obtained from field collections by R. Mamet in Mauritius, 1962. Maintained as a laboratory colony at V.B.L., selected for Silver mesonotum.
	MASC-N	Field-collected by R. Mamet in February 1965, in Mauritius. Examined after 1–2 generations of laboratory rearing.

prepare slides for microscopic examination of the male genitalia. Adult male mosquitoes were anesthetized with ether and examined under the dissecting microscope. Iris scissors were used to cut off the abdomen at about the middle of segment V. This allowed a surface for easy handling of the specimens with forceps without fear of damaging the genitalia. The genitalia were then transferred to KOH-TSP solution (50 gm KOH and 1 gm TSP in 500 ml distilled water) preheated to 50° C in the oven. The solution was covered and placed in the oven for 30–40 minutes. Next, the genitalia were transferred to preheated distilled water (50° C) and kept in the oven for 1–2 hours. They were then transferred to 75 percent EtOH at room temperature for 1 hour and then to 95 percent EtOH at room temperature for 30 minutes. The specimens were placed in clove oil at room temperature for 12–24 hours. The specimens were then put in a drop of clove oil on a slide and minuten pins were used to dissect out the genitalia from the VIIIth abdominal segment.

The specimen was placed ventral side up in a drop of euparal vert on a slide. The slide was placed in the oven at 50° C for 30 minutes to allow the euparal to set. Upon removal from the oven a coverslip with a drop of euparal vert was inverted on the specimen and the finished slide was returned to the oven for 24 hours. The slides could be used after 24 hours but could not safely be placed in a vertical position for at least 5–6 days. The slides were observed with a compound light microscope.

Figure 1 is a schematic view of the male terminalia of *A. aegypti*. It illustrates the main components of the terminalia as seen from the ventral aspect and gives the terminology used in this study.

RESULTS. Figure 2 shows schematic views of the ventral aspect of the male terminalia of *A. aegypti* and *A. mascaren-*

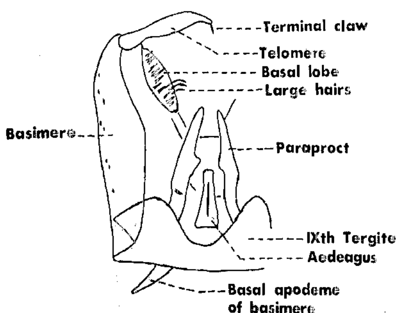

Fig. 1.—Schematic view of the ventral aspect of the male terminalia of *A. aegypti.*

sis. Figure 3 (A, B) shows photographs of *A. aegypti* and *A. mascarensis* genitalia. At least two major differences are immediately apparent. The telomeres in *A. aegypti* are curved whereas in *A. mascarensis* they are hooked. The two or three large hairs on the basal lobes are also different. In *A. aegypti* these hairs are curved and bent anteriorly. In *A. mascarensis* these large hairs have a sharp twist apically and are not curved. Since these two characters are conspicuous and readily separated, they were chosen for genetic analysis in the present study. Other apparent differences which were not analyzed in the present study, but provide ample material for future study, include the shape of the basimeres, shape of the paraprocts and the number of hairs on the basal lobes.

In the seven strains of *A. aegypti* and two strains of *A. mascarensis* (Table 1) surveyed for variability, the two characters chosen were constant within each species.

Crosses and backcrosses were made with two strains, RED EYE of *A. aegypti* and MASC-O of *A. mascarensis*. For the sake of simplicity these strains will be referred to as "A" for RED EYE and "M" for MASC-O. In all crosses the female is listed first.

Figure 3 (C, D) shows photographs of the male genitalia of F_1 males from reciprocal crosses between A and M. It is readily discernible that the structures (shape of telomeres and shape of large hairs on the basal lobes) are neither *aegypti*-like nor *mascarensis*-like, but are intermediate between the two.

It was hypothesized that these genitalic characters are controlled by a single gene, Terminalia (*T*), which shows incomplete dominance. To test this hypothesis back-

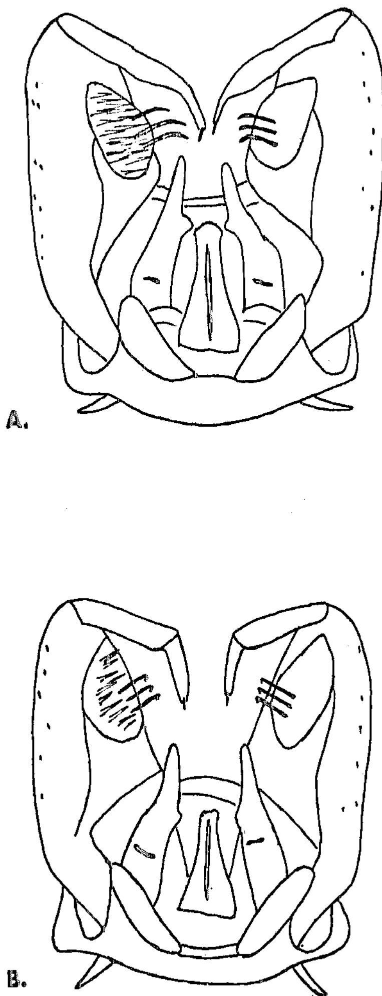

Fig. 2.—A. Ventral view of *A. aegypti* male terminalia.
B. Ventral view of *A. mascarensis* male terminalia.

crosses of F_1's to the two parental species were made. Backcross progeny were scored with regard to the chromosome markers (*re, s, blt*) and for type of telomere and type of large hairs on the basal lobes.

Genitalia of backcross progeny could readily be divided into three types: *aegypti-*

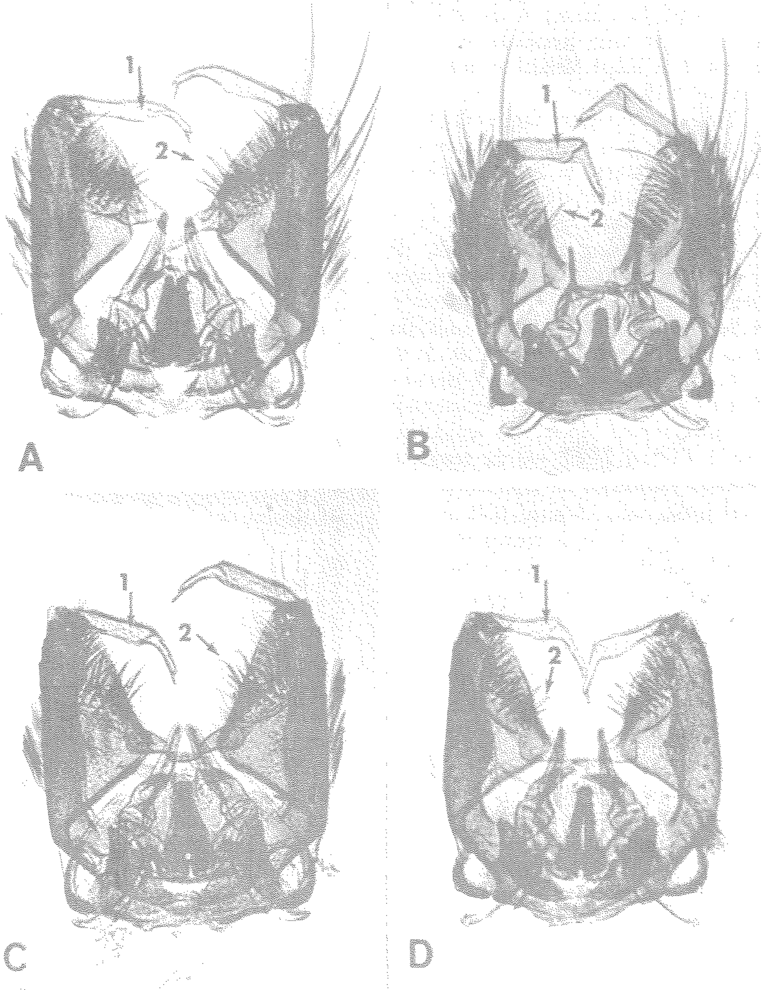

Fig. 3.—Photographs of ventral aspect of male genitalia.
A. *Aedes aegypti*
B. *Aedes mascarensis*
C. F_1 hybrid (*A. mascarensis* ♀ x *A. aegypti* ♂)
D. F_1 hybrid (*A. aegypti* ♀ x *A. mascarensis* ♂)
Arrow 1. Telomere
Arrow 2. Large hairs on basal lobes

like (*TT*), *mascarensis*-like (T^mT^m) and intermediate (TT^m), resembling the F_1 hybrids. Although occasional specimens presented problems in classification, the majority of specimens could be segregated without difficulty. This type of segregation would be expected if the characteristics of the male terminalia were controlled by a single Mendelian factor with incomplete dominance.

Table 2 summarizes the results of the backcrosses. It is evident that Mendelian segregation occurs. In the cross F_1(MxA) xA, 65 had telomeres resembling the *aegypti* parent and 50 resembling the F_1 hybrid. Following the hypothesis, this cross was TT^mx*TT* and the progeny should be 1 *TT*: 1 TT^m. The actual results give $\chi^2=1.96$ (P=.17), indicating that these data fit the hypothesis. In the cross F_1 (MxA) x M, 38 had telomeres resembling the F_1 hybrid and 37 resembled *A. mascarensis*. Here, the expectation was 1 TT^m: 1 T^mT^m and the results are essentially identical to expectation. Parallel results were obtained with the large hairs. In 3 of the 4 crosses where the female was the heterozygous parent, a 1:1 segregation ratio was evident. In the cross which failed to fit, F_1 (AxM) x A, all progeny were classified as TT^m. It was expected that the 79 progeny would give a ratio of 1 *TT*:1TT^m. No explanaation of this departure is apparent, although one might guess that the results are due to an error in classification rather than a flaw in the basic hypothesis.

Results were quite different when the male was the heterozygous parent; in the first cross of Table 2, A x F_1(AxM), 136 of the progeny were TT^m and 17 were *TT* for telomeres. These results would be expected if the gene *T* is linked to the locus for sex determination. The first cross would then be $\frac{mT}{mT} \times \frac{MT^m}{mT}$ and the male progeny from this cross would be $\frac{MT^m}{mT}$. The 17 individuals that did not fit the expectation would represent cross-

Table 2.—Segregation for genital morphology and sex (linkage group I) in backcrosses.

Type of cross			Expected segregation **			Type of male genitalia *							
						Telomere				Large hairs			
						No. observed			%	No. observed			%
Female	Male	No. progeny	*TT*	TT^m	T^mT^m	*TT*	TT^m	T^mT^m	departure	*TT*	TT^m	T^mT^m	departure
A	F_1(A x M)	153	..	153	..	17	136	..	11	31	122	..	20
A	F_1(M x A)	72	72	..	..	72	..	..	0	72	..	..	0
F_1(A x M)	A	79	39.5	39.5	..	..	79	..	50	..	79	..	50
F_1(M x A)	A	115	57.5	57.5	..	65	50	..	13	64	51	..	11
M	F_1(A x M)	92	..	..	92	..	9	83	10	..	..	92	0
M	F_1(M x A)	148	..	148	..	..	148	..	0	..	134	14	9
F_1(A x M)	M	231	..	115.5	115.5	..	133	98	8	..	116	115	0
F_1(M x A)	M	75	..	37.5	37.5	..	37	38	0	..	43	32	7

* *TT* = resembling *A. aegypti*; T^mT^m = resembling *A. mascarensis*; TT^m = resembling F_1 hybrid.
** Based on assumption of complete linkage between sex and the genitalic character.

overs, $\frac{MT}{mT}$. Figures for telomere segregation give a crossover rate of 11 percent, whereas results for large hairs give 20 percent.

The results from the second cross of Table 2 also fit the hypothesis of sex-linkage. Hypothetical genotypes of parents were $\frac{mT}{mT} \times \frac{MT}{mT^m}$ and male offspring would be expected to be $\frac{MT}{mT}$. No crossover individuals were observed among the 72 offspring.

Crosses 5 and 6 of Table 2 also gave results consistent with the hypothesis of sex-linkage. In cross 5 male offspring were expected to be $\frac{MT^m}{mT^m}$. Considering telomere type, the 92 offspring were 9 TT^m and 83 T^mT^m, for a crossover rate of 10 percent. Considering the large hairs, all 92 were T^mT^m, and no crossovers were detected. In cross 6 male offspring were expected to be $\frac{MT}{mT^m}$. Of 148 offspring, all had telomeres of TT^m, while 134 had large hairs of TT^m. The crossover rate here is 0 percent for telomere and 9 percent for large hair.

The data indicate that T is not at the sex locus. Recombination between T and m is evident in crosses 1, 5 and 6 of Table 2. The data obtained from telomeres and from large hairs are very similar. They are virtually identical in crosses 2, 3 and 4 of Table 2. The two characters are probably controlled by the same locus. The authors are inclined to believe that the evidence for independent segregation in crosses 1, 5, 7 and 8 of Table 2 is inconsequential as this may be due to classification difficulties. The data would seem to indicate a distance of approximately 10–11 units from sex-T. Further studies using other linkage group 1 markers are in progress to substantiate the position of T.

As a further check on the sex-linkage hypothesis, segregation was observed between the T locus and the chromosome markers in *A. aegypti*. This was possible only in backcrosses of F_1's to *A. aegypti*, the first four crosses in Table 2. Table 3 shows the analysis for linkage between T and markers on autosomal linkage group 2 (spot abdomen, s) and group 3

Table 3.—Analysis for linkage between T and markers on linkage group II (spot abdomen) and III (black tarsi) in backcrosses of F_1 hybrids to *A. aegypti*.

		No. individuals (N = 419) of phenotype				Chi-square analysis *	
		Wild type		Mutant (s or blt)			
Linkage analysis	Segregation	TT	TT^m	TT	TT^m	Independent assortment	Complete linkage
Expected	Linkage complete	..	209.5	209.5	..	..	..
	Independent assortment	104.5	104.5	104.5	104.5	..	..
Linkage group II spot abdomen	Observed–telomere	58	159	96	106	49.7	237.7
	Observed–large hairs	60	157	107	95	46.1	218.3
Linkage group III black tarsi	Observed–telomere	52	165	102	100	61.5	216.6
	Observed–large hairs	62	155	105	97	42.1	225.3

* Chi-square for 3 degrees of freedom and P = .05 is 7.815.

Table 4.—Segregation for genital morphology and red eye (linkage group I) in male offspring of backcrosses of F_1 hybrids to *A. aegypti*.

Type of cross				No. individuals						
				Non-red		Red				
Female	Male	No. progeny	Character segregating *	TT	TT^m	TT	TT^m	% Red eye	% TT^m	Crossover % and remarks
A	F_1(A x M)	135	Expected	..	153	..	..	0	100	sex–red = 9
			Telomere	17	122	..	14	9	89	sex–T = 11 or 20
			Large hair	26	113	5	9	9	80	....
A	F_1(M x A)	72	Expected	..	..	72	..	100	0	sex–red = 17
			Telomere	12	..	60	..	83	0	sex–T = 0
			Large hair	12	..	60	..	83	0	....
F_1(A x M)	A	79	Expected	..	39.5	39.5	..	50	50	red–T = 50
			Telomere	..	40	..	39	49	100	error, due to
			Large hair	..	40	..	39	49	100	difficulty in separation
F_1(M x A)	A	115	Expected	..	57.5	57.5	..	50	50	red–T = 39–40
			Telomere	27	31	38	19	49	44	....
			Large hair	26	32	38	19	49	43	....

* Expected figures based on assumption of complete linkage between sex, red eye and genitalic morphology.

(black tarsi, *blt*). The χ^2 values for independent assortment are high, possibly due to error in classification of genitalia type. At any rate it seems reasonable to suspect that the *T* locus shows independent assortment with both autosomal linkage groups. Since there are only 3 linkage groups in *A. aegypti*, these data provide additional evidence for the hypothesis that *T* is in linkage group 1.

Further evidence for this hypothesis is provided in Table 4. This table shows segregation of *T* and red eye *(re)* in backcrosses of F_1 hybrids to *A. aegypti*. The crossover values from some crosses seem to indicate that *T* is to the right of sex, on the opposite side from red eye.

Figure 4 is a crossing scheme to demonstrate the inheritance of *T*.

DISCUSSION. The data generally fit the hypothesis that the morphology of the telomeres and large hairs on the basal lobes of the male genitalia is controlled by a single gene, Terminalia *(T)*, which shows incomplete dominance and is located in linkage group 1. More data are needed before one can give the position of *T* on chromosome 1 with confidence. Crossover studies using bronze and white eye, markers on chromosome 1, are needed to substantiate the position of *T*.

Perhaps the *T* locus exists in other species of mosquitoes. Perry (1950), in conducting hybridization studies with *Aedes hebrideus* and *A. pernotatus*, made the following backcross: female F_1 (female *A. hebrideus* x male *A. pernotatus*) x male *A. pernotatus*. All the males re-

P_1 : $\frac{m\ T^m}{m\ T^m}$ x $\frac{M\ T}{m\ T}$

F_1 : $\frac{m\ T^m}{m\ T}$ and $\frac{M\ T}{m\ T^m}$

Backcrosses:	Expected male progeny:
1. $\frac{m\ T^m}{m\ T}$ x $\frac{M\ T}{m\ T}$	$\frac{M\ T}{m\ T^m}$ and $\frac{M\ T}{m\ T}$
2. $\frac{m\ T^m}{m\ T}$ x $\frac{M\ T^m}{m\ T^m}$	$\frac{M\ T^m}{m\ T^m}$ and $\frac{M\ T^m}{m\ T}$
3. $\frac{m\ T}{m\ T}$ x $\frac{M\ T}{m\ T^m}$	$\frac{M\ T}{m\ T}$
4. $\frac{m\ T^m}{m\ T^m}$ x $\frac{M\ T}{m\ T^m}$	$\frac{M\ T}{m\ T^m}$

Fig. 4.—Crossing scheme to demonstrate inheritance of *T*.

covered from this cross had *pernotatus*-like genitalia. His data seem to indicate that *pernotatus*-like genitalia is determined by a sex-linked dominant factor.

Dobrotworsky (1955) conducted cross breeding experiments within the *Culex pipiens* group. The type of males he obtained in backcrosses of *Culex molestus* x *C. globocoxitus* hybrids to *C. molestus* seem to indicate sex-linkage for genital morphology. The results hold for crosses with *C. fatigans* and *C. globocoxitus.*

Among the culicine species, extensive genetic studies have been conducted only with *Culex pipiens* and *Aedes aegypti.* Craig and Hickey (1967) have pointed out similarities in chromosome 1 of these two species. The gene for sex determination is on chromosome 1 in both species. Among 5 eye color genes in *A. aegypti* 3 are sex-linked. Among 3 in *C. pipiens,* 2 are sex-linked. The white eye of *C. pipiens* and the red eye of *A. aegypti* are each about 7 units from the locus for sex determination. One may suspect that the chromosomes of the two species are homologous for much of their length.

Perhaps *Culex* and *Aedes* are similar with respect to the *T* locus. This might be discovered in crosses between *Culex pipiens pipiens* and *Culex fatigans.* These show distinctive differences in male genitalia; moreover, they can be hybridized. Numerous marker genes are available in *Culex pipiens* and a genetic analysis of genitalic differences, similar to the present study, should provide no difficulties.

CONCLUSIONS. 1. *A. aegypti* and *A. mascarensis* are closely related species which differ in the shape of the telomeres and large hairs on the basal lobes of the male genitalia.

2. The structure of telomeres and large hairs remains constant among different strains of *A. aegypti* and *A. mascarensis.*

3. These genital characters appear to be controlled by a single gene, Terminalia *(T),* which shows incomplete dominance and is located in linkage group 1, possibly to the right of the sex locus.

Literature Cited

Craig, G. B., Jr. and W. A. Hickey. 1967. Genetics of *Aedes aegypti. In:* J. W. Wright and R. Pal (ed.). Genetics of insect vectors of disease. Amsterdam: Elsevier Press, pp. 67–131.

Craig, G. B., Jr. and R. C. VandeHey. 1962. Genetic variability in *Aedes aegypti* (Diptera: Culicidae). I. Mutations affecting color pattern. Ann. Entomol. Soc. Amer. 55:47–58.

Dobrotworsky, N. V. 1955. The *Culex pipiens* group in southeastern Australia. IV. Crossbreeding experiments within the *Culex pipiens* group. Proc. Linn. Soc. N. S. W. 80:33–43.

Edwards, F. W. 1941. Mosquitoes of the Ethiopian Region. Vol. III. Culicine Adults and Pupae. London: British Museum (Natural History). 499 pp.

Hartberg, W. K. and G. B. Craig, Jr. 1968. Reproductive isolation in *Stegomyia* mosquitoes. I. Sexual isolation between *Aedes aegypti* and *A. mascarensis.* Ann. Entomol. Soc. Amer. 61(4):865–870.

Hartberg, W. K. and G. B. Craig, Jr. 1970. Reproductive isolation in *Stegomyia* mosquitoes. II. Hybrid breakdown between *Aedes aegypti* and *A. mascarensis.* Evolution 24(4): 692–703.

Hodapp, C. J. and J. C. Jones. 1961. The anatomy of the adult male reproductive system of *Aedes aegypti* (Linnaeus) (Diptera, Culicidae). Ann. Entomol. Soc. Amer. 54:832–844.

Mattingly, P. F. 1966. The culicine mosquitoes of the Indomalayan area. Part VI. London: British Museum (Nat. Hist.)

Mattingly, P. F. and L. J. Bruce-Chwatt. 1954. Morphology and bionomics of *A. (S.) pseudoafricans* Chwatt, with some notes on the distribution of the subgenus *Stegomyia* in Africa. Ann. Trop. Med. Parasitol. 48:183–193.

McClelland, G. A. H. 1962. A contribution to the genetics of the mosquito *Aedes aegypti* (L.) with particular reference to factors determining colour. Ph.D. thesis, Univ. London. 313 pp.

McClelland, G. A. H. 1967. Speciation and evolution in *Aedes. In:* J. W. Wright and R. Pal (ed.). Genetics of insect vectors of disease. Amsterdam: Elsevier Press, pp. 277–311.

McClelland, G. A. H. and R. Mamet. 1962. *Aedes aegypti* (L.) and *Aedes mascarensis* MacGregor in Mauritius: A case of gene survival following species eradication? Nature 195:965.

Perry, Wm. J. 1950. Biological and crossbreeding studies on *Aedes hebrideus* and *Aedes pernotatus.* Ann. Entomol. Soc. Amer. 43: 123–136.

Spielman, A. 1964. The mechanics of copulation in *Aedes aegypti.* Biol. Bull. 127(2): 324–344.