

INCIDENCE OF GENETIC MUTATIONS IN
*CULEX PIFIENS*¹

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INTRODUCTION. Considerable interest in the genetics of mosquitoes has been seen in the past ten years. Some of the interest arose from attempts to understand variations in vector ability and other behavioral traits noted among mosquito populations. This interest was further stimulated by the desire to understand the development of resistance to insecticides by those species known to be vectors of disease. This interest in mosquito genetics has been sustained by the continual discovery of new mutant forms. These mutants have proven to be valuable not only in understanding the above phenomena but also in designing programs for biological control.

The greatest number of mosquito mutants has been discovered in the yellow fever mosquito, *Aedes aegypti*. Within the short span of the past ten years, recognition of the number and variety of mutant forms in this species has been somewhat comparable to the early discoveries of mutant forms in *Drosophila melanogaster*. It has been shown by Craig and his coworkers that approximately 87 mutants are now known in *A. aegypti* and that each mosquito of this species carries an average load of 0.52 to 2.96 visible mutations. The work of the past ten years with *A. aegypti* has been capably and thoroughly summarized in a recent publication by Craig and Hickey (1967).

To date, fewer mutants are known for *Culex pipiens*. Most of these are listed in Table 1. Laven and Kitzmiller have carried out most of the studies. It can be noted that both spontaneously occurring and radiation-induced mutants have been described. The greatest number of radia-

tion-induced mutants have been those affecting the palpi and wings, while spontaneously occurring mutants have been noted for most of the body parts. Whether some of the mutants were spontaneously occurring or radiation-induced is a difficult, if not impossible, question to answer. At present, one can say that there are approximately an equal number of radiation-induced and spontaneously occurring mutants known in *C. pipiens*. In addition to the 34 morphological mutants listed in Table 1, Barr (personal communication) has found at least ten others, namely, divided eye, enlarged tergum, fused abdomen, mottled (two types), dwarf (two types), antennaless, pigmented pupal paddle and reflected pupal antenna. Some of these are still under study. A number of physiological or behavioral mutants, such as resistance to DDT, autogeny and stenogamy have also been described by various authors.

While Craig and his coworkers have obtained an estimate of the mutation load carried by *A. aegypti*, no such estimate of the incidence or frequency of mutations in *C. pipiens* is known. The purpose of the present work is to supply such an estimate for a sample of one natural population. The major portion of this work was carried out 5 years ago in Germany.

MATERIALS AND METHOD. The method employed was an inbreeding program designed to reveal mutations carried in the heterozygous condition. It was similar to the programs used in the analysis of genetic variability of *Drosophila* species (Spencer, 1947) and *A. aegypti* (Craig *et al.* 1961). Essentially, the program consisted in examining the progeny of a number of F₁ sib-matings. Egg rafts collected from paternal females were placed in separate containers for hatching and the rear-

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ing of larvae until pupation. For emergence and random mating of F₁ adults, all pupae obtained from a single egg raft were placed in the same cage since multiple fertilization rarely occurs in *C. pipiens* (Kitzmilller, 1958). It is probable that a hormone from the male accessory gland prevents multiple fertilization in this

species in a manner even more effective than that demonstrated for *A. aegypti* (Spielman *et al.* 1967). Egg rafts obtained from the F₁ adults were again placed in separate containers and the hatching F₂ progeny were reared until they were adults. These were examined for any departures from the normal appearance of

TABLE 1. —Morphological mutations in *Culex pipiens*.

Mutant designation *	Spontaneously occurring mutant	Obtained from irradiated mosquitoes	Reference
Eyes			
red (<i>r</i>)		X (?)	Wild (1963)
ruby (<i>ru</i>)	X		Iltis. <i>et al.</i> (1965)
white (<i>w</i>)	X		Gilchrist & Haldane (1947)
Antenna and Palp			
broken antenna (<i>ba</i>)	X		VandeHey (1964)
clubbed palp (<i>kps</i>)		X (?)	Laven (1955)
clubbed palp (<i>kps</i> ?)		X	Kitzmilller (1958)
clubbed palp, asymmetrical (<i>Kpu</i>)		X	Laven (1957)
clubbed palp, asymmetrical (<i>Kpu</i> ?)		X	Kitzmilller (1958)
extended patch of scales (<i>Pff</i>)		X	Laven (1957)
four-jointed palp (<i>Aj</i>)		X	Kitzmilller (1958)
reduced antenna and palp (<i>Rap</i>)		X	Laven (1957)
reduced antenna (<i>Ant</i>)	X (?)		Laven (1967)
Wings			
anal vein interrupted (<i>ani</i>)	X		VandeHey (1964)
closed radial cell (<i>R₂</i>)		X	Kitzmilller (1958)
double fusion of veins		X	Laven (1957)
fusion of veins R ₃ & R ₄₊₅ (<i>var</i>)		X	Laven (1957)
interrupted veins R ₂ & M ₃		X	Laven (1957)
interrupted vein M ₃ (<i>mi</i>)	X		VandeHey (1964)
scale row between R ₁ & R ₂ (<i>Sch</i>)		X	Laven (1957)
scale row between R ₁ & R ₂ (<i>sch</i>)		X	Kitzmilller (1958)
scale row in cubital area (<i>cus</i>)		X	Kitzmilller (1958)
short wing (<i>kff</i>)	X		Laven (1957)
shortened (<i>Kuf</i>)	X		Laven (1957)
spot of scales on r-m (<i>spot</i>)		X	Kitzmilller (1958)
Thorax			
golden scales on mesonotum (<i>G</i>)	X		McClelland (1967)
Abdomen			
DV/D ratio	X		Barr & Kartman (1951)
scale color variation	X		Callot (1947)
Larva			
black head capsule (<i>Bl</i>)	X		VandeHey (1967)
curved antenna (<i>la</i>)	X		Barr & Myers (1966)
green (<i>g</i>)	X		Huff (1929)
melanotic (<i>mel</i>)	X		Laven & Chen (1956)
yellow (<i>y</i>)	X		Ghelelovitch (1950)
Other			
intersexuality (<i>zwi</i>)	X		Laven (1955)
gynandromorphism (<i>gyn</i>)	X		Laven (1957)

* Symbols, when given, are those recorded in the literature; in several cases, indicated by a question mark, the symbols listed are tentative.

the imago. Tests for heritability of the abnormal phenotypes consisted in recovery of identical phenotypes among their progeny after sib-mating for two additional generations.

Throughout the work, standard culture techniques were employed. Larvae were reared in large glass finger bowls and fed a mixture of yeast and ground dog biscuits. The larvae were transferred to fresh water every 2 or 3 days. On this regimen, pupation occurred approximately 15 days after hatching. Cages for emergence and maintenance of the adults were pint jars $\frac{1}{4}$ -filled with water and covered with netting. Adults were allowed to feed on sugar cubes. No blood meals were supplied since it was desirable to work with an autogenous population.

RESULTS. In August, 1963, 15 inseminated females were collected from a home in Bodenheim, Germany. F_1 progeny were obtained from each female. Two of the females were probably anautogenous since egg rafts were not obtained from any of their F_1 progeny. A total of 256 F_2 rafts were obtained from the remaining 13 parental females. The analysis of these is shown in Table 2. From the egg rafts obtained, an average of ten per P_1 female ($N = 129$) were randomly selected for rearing and examination of the F_2 adults.

As Table 2 shows, 20 different phenotypic abnormalities were noted in the examination of the 4434 F_2 adults. Six other abnormalities, not listed in the table, were thought to be non-genetic since they involved, at most, the unilateral loss of only small portions of a tarsus, antenna or palp. Time and space were available to test the heritability of eight of the more promising abnormalities (starred items in the table).

On the basis of recovery of identical phenotypic expressions within the next two generations, only two of the tested phenotypes proved to be genetic. Both of these were wing mutants, showing modifications of the veins. One, an interruption of a medial vein, M_3 , was isolated from culture #13.8c; the other, an interruption or shortening of the anal vein, was isolated

from culture #15.8b. It will be noted that both phenotypic expressions were also found among the progeny of two other parental lines, culture #24.8 and 31.8c respectively. In addition to these four mutant recoveries, two other mutants were discovered in the F_3 and F_4 generations. A larval mutation causing increased pigmentation of the head capsule was obtained from the progeny of the notched-winged male (#25.8b) and an antennal mutation showing partial to complete loss of the female flagellum was obtained from a line selected for increased white scaling on the female palp (#27.8).

A preliminary description of these mutants was given by VandeHey (1964). The larval head capsule mutant, *Bl*, has been described and analysed in detail by VandeHey (1967). It was shown to be inherited as a sex-linked factor with complete dominance in the male and incomplete dominance in the female. A more complete description of the other mutants follow here.

Interrupted medial vein, M_3 , (mi) was somewhat similar in expression to a mutant described by Laven (1957) as *Rückbildung der Adern m_3 und r_2* . In *mi* only the medial vein, M_3 , was affected; its proximal end was not jointed to M_{1+2} . Usually only the distal half of the vein was present. There was some variability of expression from a barely perceptible interruption to almost complete absence of the vein (Fig. 1-3). Penetrance was incomplete. In a stock under selection pressure for three generations, 90 percent of the males and only 50 percent of the females showed the trait. The mutant phenotype was not present in the F_1 progeny of an outcross to another inbred line. In the F_2 of these outcrosses, only one out of seven males and one out of ten females showed the trait. In backcrosses, the ratio of normal to mutant phenotypes was 1:3 among the males and a little over 3:1 among the females. Thus, the mode of inheritance for this mutation appears to be recessive with incomplete, sex-influenced penetrance.

Interrupted anal vein (ani) showed partial to complete absence of the anal vein in

TABLE 2.—Abnormalities recorded among the F₂ progeny of 13 autogenous females of *Culex pipiens*.

Culture number	Number of F ₂ egg rafts		Number of F ₂ adults examined	Abnormalities	No. affected individuals
	Obtained	Examined			
13.8a	23	10	311	hook on terminal antennal segment	1 ♀
13.8b	6	6	308	* short setae on distal antennal segment fusion of several antennal segments	3 ♀ 2 ♀ ♀ 15 ♀ & ♂
13.8c	14	13	560	* interrupted vein M ₃ delta-shaped wing	1 ♀ 8 ♂
15.8b	14	10	211	* shortened anal vein	8 ♂
17.8	4	4	158	bristle-like scales on abdomen	3 ♀ ♀
18.8a	10	10	405	reduced white scales on dorsum of abdomen	1 ♀
				reduced white scales on palp	4 ♀
				* setae appressed to antennal flagellum	2 ♀ ♀
				* antennal segments fused	4 ♀ ♀
				* increased white scales on palp	4 ♀ ♀
24.8	42	14	634	bowled legs, wings curved over side of abdomen	1 ♂
				interrupted vein M ₃	2 ♀ ♀
				* shortened antenna	3 ♀ ♀
25.8a	19	9	197	twisted proboscis	1 ♀
25.8b	19	10	426	* notched wing (trailing edge, unilateral)	1 ♂
27.8	26	10	384	* increased white scales on palp	1 ♂
31.8a	36	12	393		24 ♀
31.8b	24	11	179	lethals (?)	
31.8c	19	10	268	shortened anal vein small, upturned wings	1 ♀ 1 ♀

* Abnormalities which were tested for inheritance.

the male (Fig. 4-6). The mode of inheritance could not be determined because of associated lethal factors. It was first isolated as eight males in three out of ten families from one of the parental females. In the next generation of inbreeding, 92.5 percent of the hatched larvae ($N = 424$) died as second or third instars. Half of the surviving males ($N = 16$) showed the trait; all the females ($N = 16$) were nor-

mal. In the next generation all eggs were unembryonated.

Broken antenna (ba), isolated in the F_3 , showed partial to complete absence of the antennal flagellum in the female. Penetration was generally very low and the mutant was discarded after four generation of inbreeding.

On the basis of recovery of the two tested mutants and two others of similar

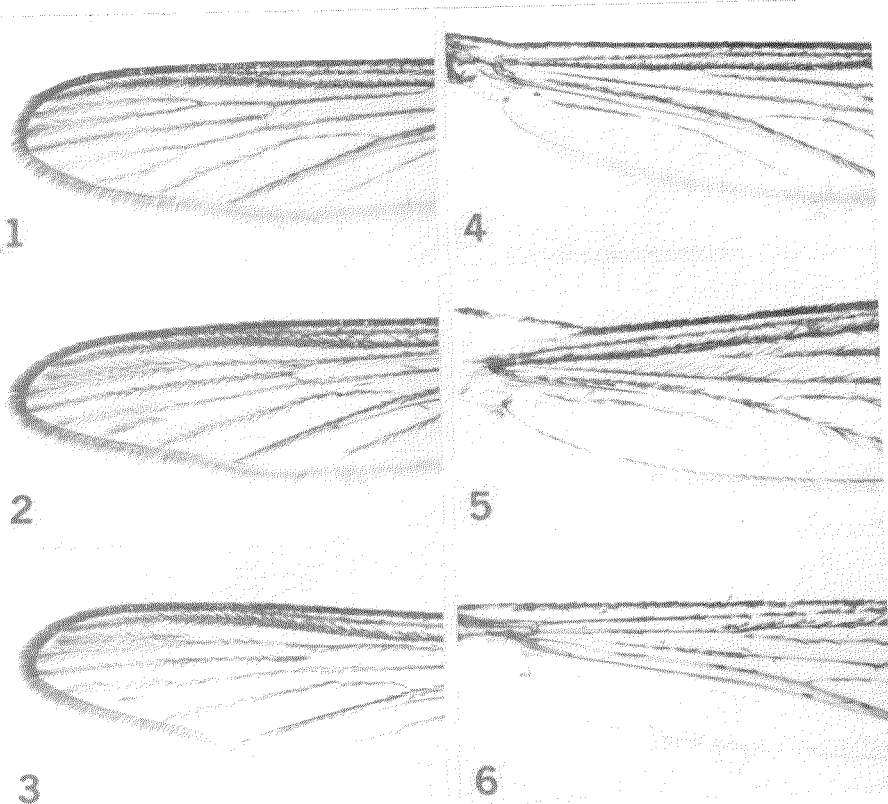


FIG. 1.—Normal wing.

FIG. 2.—Wing of mutant, *mi*, showing slight interruption of medial vein, Ms.

FIG. 3.—Wing of mutant, *mi*, showing pronounced interruption of medial vein, Ms.

FIG. 4.—Normal wing.

FIG. 5.—Wing of mutant, *ani*, showing partial reduction of anal vein.

FIG. 6.—Wing of mutant, *ani*, showing pronounced reduction of anal vein.

phenotype in the F_2 , the present analysis indicates that the sample of 13 inseminated females from Bodenheim had a mutation frequency of 0.32 per pair or 0.16 per mosquito. If the two mutants recovered in the F_3 and F_4 are also considered, the mutation frequency for this population was 0.48 per pair or 0.24 per mosquito.

DISCUSSION. Recovery of the mutants is dependent on the laws of probability. In the first place, recovery of a recessive is dependent on the number of F_1 sib-matings which produce progeny for examination and, secondly, it is dependent on the number of progeny produced by each sib-mating. The chance of a successful recovery is the product of these two probabilities. In the present work, mutants appeared only in those lines with ten or more F_1 sib-matings producing an average of at least 20 progeny per mating. Thus the chances of recovery of recessive mutants in these cases were nearly 94 percent or higher. It is quite likely that all the mutations carried by these parental females were actually recovered. However, one of the parental lines had only four sib-matings and another only six, giving probabilities of only 68 percent and 82 percent, respectively, for mutant recovery. Some mutants may have been missed in these families. For this reason, the mutation frequency of 0.16 per mosquito is considered minimal. However, since no mutants were actually recovered in these latter families, there is no basis on which to estimate the number of mutants which might have been recovered if more sib-matings had been obtained for examination. Nevertheless, the recovery of additional mutants in later generations indicates that the frequency value of 0.24 may be more realistic for this population.

It is interesting to note the similarity of some of the mutant phenotypes isolated so far in *C. pipiens*. White eye (*w*) which had been lost after the initial work of Gilchrist and Haldane has been recovered several times (Kitzmiller, in 1953; Laven, 1964). Some of the palpal mutants isolated independently by Laven and Kitzmiller are quite similar, e.g., *kps* and *Kpu*. Inter-

rupted medial vein (*mi*) described here and closed radial cell (R_2) described by Kitzmiller are both expressed in a single phenotype isolated by Laven (double fusion of veins). Through the courtesy of Dr. Iltis, the author observed variations in the pigmentation of some preserved larvae collected in Oregon which seemed similar to the black larva mutant (*Bl*). Tests for allelism seem to be indicated in these cases.

The data so far obtained indicate that *C. pipiens* is not as variable in morphological characteristics as is *A. aegypti*. Craig and Hickey (1967) list mutation frequencies as high as 2.72 and 2.96 for several populations of *A. aegypti* from Kenya, Africa. On the other hand, some feral populations of the same species from Congo, Nigeria and Ghana gave frequencies of 0.72, 0.84 and 0.52, respectively. Even these latter frequencies are twice to three times the value found here in *C. pipiens*. It is perhaps too early, however, to make valid comparisons between the two species. Other populations of *C. pipiens* should be analysed.

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THE RAMP-TRAP, AN UNBAITED DEVICE FOR FLIGHT STUDIES OF MOSQUITOES

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The study of the flight paths of mosquitoes has been hampered in the past by lack of suitable traps. The most commonly used method for actively sampling flying populations is the truck trap of Chamberlin and Lawson (1945). This has been modified in various ways, e.g., Sommerman and Simmet (1965), Loy *et al.* (1968), and has been profitably used by Bidlingmayer (1964, 1966) to study the flight activity of *Aedes taeniorhynchus* (Wiedemann) in relation to the lunar cycle. It takes a linear sample of a very large volume of air and hence provides large catches, but its use is strictly limited to motorable terrain and it can give only

marginal information on vertical distribution. Moreover, in common with other active sampling methods, including suction traps and the rotating net traps of Chamberlin and Lawson (1945), it is impossible to obtain any directional information from its use.

The alternative approach is to adopt passive sampling methods in which the mosquitoes enter the trapping area whilst in flight and are trapped or not according to their reactions. Examples of these are sticky traps, as used by Gordon and Gerburg (1945) and Provost (1960), the stationary traps of Nielsen (1960), and the open net-traps tested by Colless (1959) in