Genet. Res., Camb. (1967), **10**, *pp.* 205–217
With 2 plates
Printed in Great Britain

Genetic studies on mutations in species A and B of the *Anopheles gambiae* complex*

By G. F. MASON†

*Ross Institute of Tropical Hygiene, London, W.C.*1

(*Received* 25 *May* 1967)

1. INTRODUCTION

The great impetus given by the resistance problem has resulted in mosquito genetics being one of the most rapidly growing areas of medical entomology. Resistance with its genetic basis of inheritance has underlined more than anything else the need for genetic and cytogenetic research on vector species. Despite the fact that many anopheline species have been colonized for quite some time, the genetics of them is one of the fields in which little is known. There are many taxonomic references in the literature to variations in characters but in spite of this the only genetic studies on *Anopheles* until recently were those on resistance to insecticides. At the same time as these resistance studies were taking place in *Anopheles* the main advance in mosquito genetics was taking place in the *Culicini*. Aside from resistance studies, *Anopheles* species were used only for cytogenetic work on speciation. This lack of knowledge of formal anopheline genetics, in contrast with the progress made in cytogenetics, has resulted from a number of difficulties. Perhaps the most important of these was the inability to mate single pairs and establish colonies of any mutants which were discovered. This impediment has been overcome by the development of induced mating techniques and the way is now open for the colonization of mutant strains.

In this paper the inheritance of some naturally occurring mutants in the cryptic species *An. gambiae*, species A and species B (Davidson & Jackson, 1962) and other characters are reported.

2. MATERIALS AND METHODS

The mutations employed in this study were selected from two colonies of *An. gambiae* species A and three colonies of *An. gambiae* species B (Davidson & Jackson, 1962). Mutations with the same phenotype and pattern of inheritance were discovered and isolated in each of the cryptic species.

* The author gratefully acknowledges the support given to this project by the Leverhume Foundation, Medical Research Council and the World Health Organization.

† Present address—Medical Research Council Experimental Genetic Research Unit, University College London, Wolfson House, London, N.W.1.

(i) *Species A colonies*

Lagos; a susceptible colony which has been colonized at the Ross Institute since 1951. The original material was collected around Lagos, Nigeria, and was kept in the Malaria Service's laboratory in Lagos.

Sokoto; a colony from Tungan Buzu, a village in the Sokoto region of Nigeria where DDT had been used for a number of years. When this material was collected in July 1951, the village was in a 'Pilot Project Zone' of the Mass Malaria Control Campaign, Birnin Kebbi.

(ii) *Species B colonies*

Kano; a (semi-dominant) resistant colony received in 1956 from Kano, Nigeria. This strain originated from females collected in an area around Kano where BHC had been used. It is homozygous for the semi-dominant dieldrin-resistant gene.

Somalia; a strain obtained from the 'Instituto di Malariologia E. Marchiafavo', Monticelli, Italy, in 1959. The original adults were collected around Mogadiscio in Somaliland.

Mak; an insecticide-susceptible colony collected at Makzan, Abyan, West Aden Protectorate. Eggs from females caught in the wild were received in November 1962 and a colony was formed.

(iii) *Methods*

The larval stages of these strains were kept in rooms at a temperature between 25° and 27°C. They were fed 'Farex', a baby food containing 2·5% fat, 14·2% protein, 72·7% carbohydrate, 3·6% mineral salts and 6·5% fibre. In addition a small piece of turf was added to each larval bowl as a source of micro-organisms on which the larvae also fed. The amount of light in the larval rooms was controlled by a time-switch set to give 12 hours of light per day. Adults were maintained in an insectary at a temperature of 27°C. and a relative humidity of 75%. Cages for the adults consisted of mosquito netting on a 12-inch square stainless-steel frame. A 20% sugar solution on a cotton wick was continuously provided for the males, while the females were allowed to take a blood meal twice a week from a guinea-pig anaesthetized with nembutal.

The morphological mutants recorded are the result of a search for naturally occurring mutants in species of the *An. gambiae* complex. The eggs, larvae and adults of the F_2 generation from brother-sister matings were examined for variations in colour pattern and morphological differences. Any individuals having a deviation from the normal were isolated and mated with their sibs. In addition to the single-pair analysis on field material, random searches were made on the larvae and adults of the stock strains. All those characters which reappeared in subsequent generations were classed as heritable and attempts were made to discover their heredity. All mutants were colonized, and subsequently their mode of inheritance determined by induced mating of single pairs. The mating technique which was employed is a modification of the original method devised by McDaniel & Horsdalls (1957). It was best carried out using 2- to 3-day-old males and females which had been kept

in separate cages from the time of emergence; this appeared to increase the performance of the males. The preparation of the males for mating did not involve anaesthetic. Instead they were handled by a curved vacuum micropipette affixed to their thorax or proboscis. Holding the male in this manner enables the wings and legs to be trimmed off, and the animal decapitated. After a number of males had been prepared, they were glued with a water-soluble glue, ventral side up and in a horizontal position to the lip of a petri dish. A freshly fed female was then placed on a glass plate, and the micropipette attached to her dorsal thorax in line with her body. Thus held she was then lined up with the male and held at an angle of 90 degrees or slightly less. The male was then stimulated by having his terminalia repeatedly touched with the tip of the female abdomen. Repeated stimulation in this way usually resulted in the male clasping the female very tightly, his telomeres encircling her abdomen and compressing it. During this clasping and compression the male aedeagus entered the female atrium. The whole mating process took about 20–30 seconds, after which the male released the female. It was noticed that during the terminal part of their mating process the male produced a mating plug which was sometimes seen protruding from the atrium of the female (Mason, 1964).

After mating, the females were placed in 3 inch by 1 inch glass vials and mosquito netting was stretched across the mouth and held in place by an elastic band. On the third day after feeding and mating, one-quarter of an inch of water was added to the tubes for the females to lay their eggs in. After ovipositing the females were placed in dry tubes and given another blood meal. Most females laid from one to three egg batches, each one containing 125 to 175 eggs. Additional water was then added to the egg tubes and the eggs were left to hatch. Each single egg-laying was reared in an 8 inch by 3 inch enamel bowl. After hatching had commenced the eggs and larvae were washed into a wax-paper ring floating in the bowl. This precaution prevented the unhatched eggs from being stranded and, as a result, ensured almost a complete hatch. From this point on the rearing method used for stock cultures was then followed.

3. DESCRIPTION OF MUTANTS AND OTHER CHARACTERS

(i) *Collarless* (c) *larvae*

This naturally occurring larval mutant was isolated from the Lagos strain of the A group in the *An. gambiae* species complex. It affects the development of the white pigment in the thorax of the larvae. Larvae with the wild-type allele of this mutant are readily recognized by the white pigment which is visible to the naked eye. The pigment forms a collar of various shapes on the thorax immediately behind the larval head (Plate I, Fig. 1). This collar is present in all of the larval stages and remnants of it can be seen in the cuticle on the anterior and dorsal survace of the pupal cephalothorax (Plate I, Fig. 2). The fact that this collar remains visible despite successive molts would indicate the pigment to be in the parietal layer of fat below the cuticular epidermis. The mutant larvae lack this white pigment and consequently it is not visible in the resulting pupae (Plate I, Figs. 3–4). Due to the

size and density of the collar there is a marked difference between the two phenotypes and this enables them to be separated, in both the larval and pupal stages, without the aid of a microscope. As well as lacking the collar the mutant larvae are generally a darker colour with more brown body pigment.

Reciprocal crosses between single adults of the mutant and wild-type strains were made to test for the monofactorial inheritance of this character. Some of the F_1 hybrids were inbred to obtain the F_2 generation and others were backcrossed to the mutant type. The results of these crosses are recorded in Table 1. All of the outcross matings of collarless to wild-type individuals produced only normal larvae, and all of the collarless by collarless produced only collarless individuals.

The pooled data recorded in Table 1 are in very good agreement with expectation, and there was no heterogeneity between parallel pair matings. Thus the results obtained show the mutation collarless to be inherited as a single, autosomal recessive gene with full penetrance and good viability.

Table 1. *Segregation of collarless* (c)

Parents: collarless ♂ × + (wild strain) ♀
F_1 : all +

Segregation of F_2 and backcross (10 pairs)

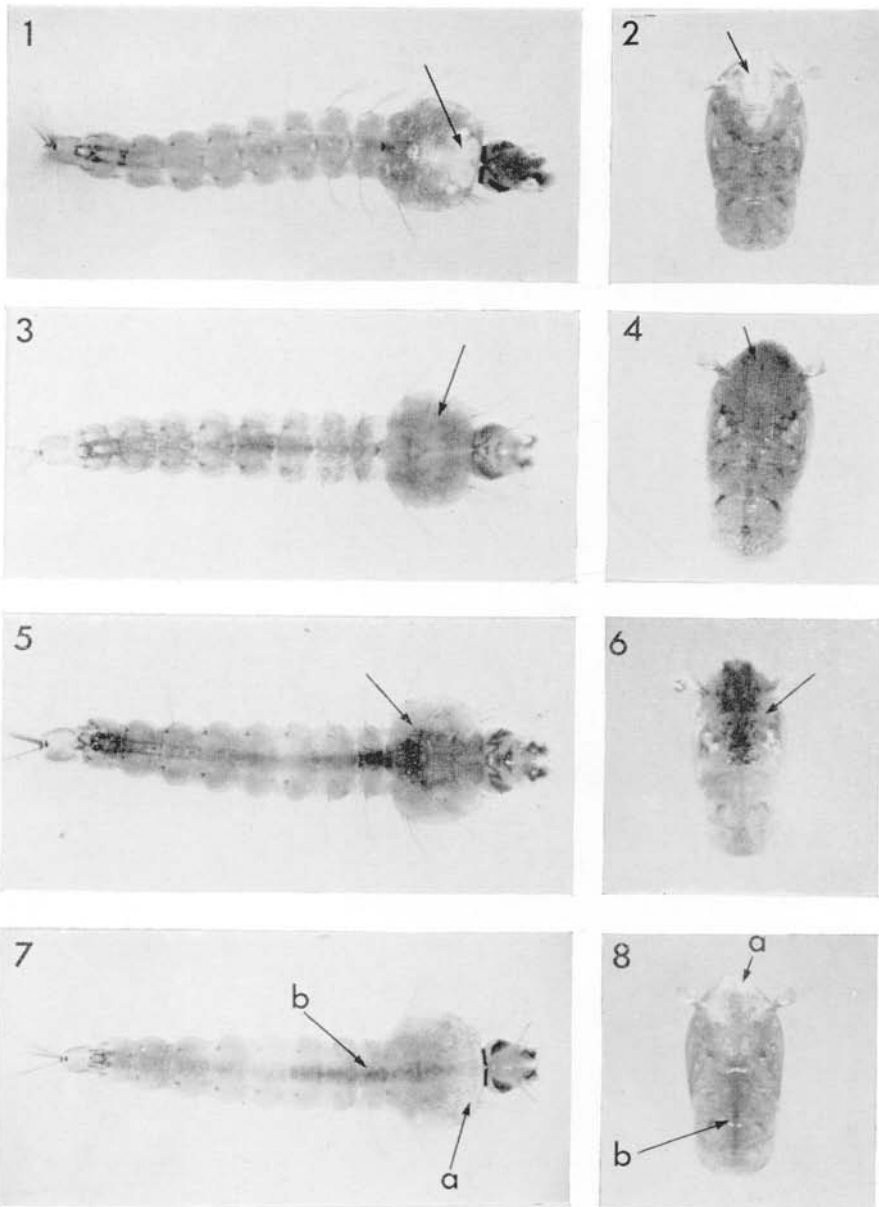
	F_2 +	Collarless
	967	329
Backcross to collarless ♂ (5 pairs)	268	261

Collarless × collarless: all collarless

A similar mutant has been isolated from the Somalia colony which is from the species B group of the complex. Its expression and inheritance are, as nearly as can be detected, identical to that found for the gene in the species A Lagos strain.

(ii) *Diamond*

This character expresses itself as a black pigmented diamond-shaped area in the dorsal thorax and first abdominal segment of the larvae (Plate 1, Fig. 5). Though present in both sexes in early larval life it is less dense in male larvae and is usually lost by the time the fourth stage is reached. Female larvae retain the black pigmented diamond all through larval life. The diamond shape is lost in the pupae but the black pigment is visible on the anterior and dorsal surface of the cephalothorax (Plate 1, Fig. 6). On occasion, there are male larvae which have a diamond as dark as the females and they also retain it in the pupae. This naturally occurring character is quite common in both species A and B of the complex. In some colonies the diamond is quite distinct in outline while in others it is more diffuse. Similarly it varies in its shape in the different colonies and sometimes appears as a triangle rather than a diamond. Strains homozygous for this character were isolated from the A group Lagos colony and the Mak colony of the B group. Duplicate experi-



EXPLANATION OF PLATES

Plate I

Fig. 1. White collar (c^+) larva (wild type).
Fig. 2. Pupa—with remnant of the white collar (c^+) on the cephalothorax.
Fig. 3. Mutant collarless (c) larva.
Fig. 4. Collarless pupa (c).
Fig. 5. Black diamond larva.
Fig. 6. Pupa with remnant of black diamond.
Fig. 7. Larva with: (a) white collar (c^+), (b) red stripe on abdomen and thorax.
Fig. 8. Pupa with: (a) white collar (c^+), (b) red stripe.

https://doi.org/10.1017/S001667230001096X Published online by Cambridge University Press

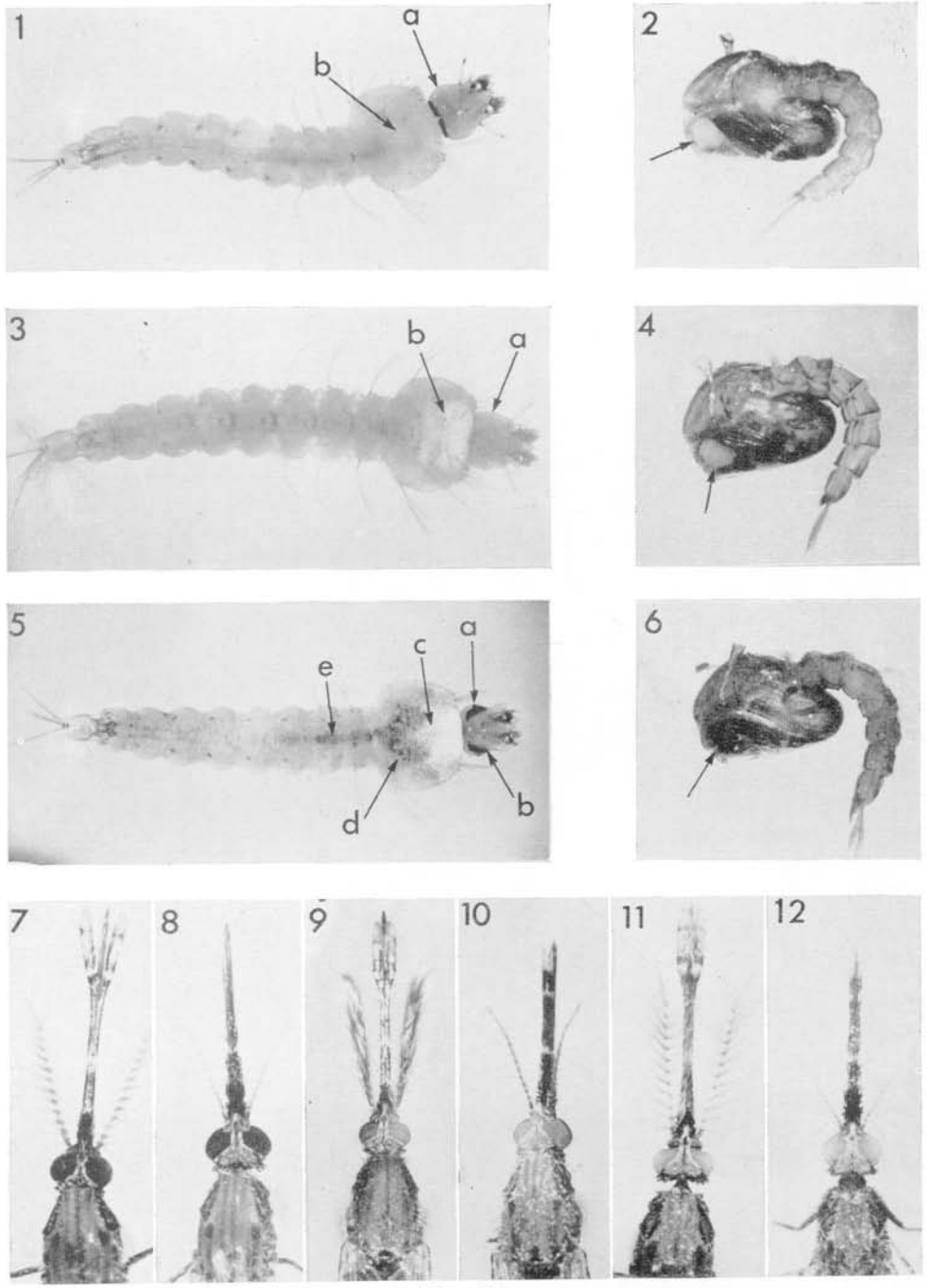


Plate II

Fig. 1. Mutant white-eye (*w*) larva: (*a*) white imago and larval eye, (*b*) no pigment in the body.

Fig. 2. White-eye (*w*) pupa.

Fig. 3. Mutant pink-eye (*p*) larva: (*a*) pink imago and larval eye, (*b*) white collar (c^+).

Fig. 4. Pink-eye (*p*) pupa.

Fig. 5. Larva with: (*a*) normal larval eye (w^+)), (*b*) developing imago eye (w^+), (*c*) white collar (c^+), (*d*) diamond, (*e*) red stripe.

Fig. 6. Wild-type eye-pupa.

Fig. 7. Wild-type eye, adult male.

Fig. 8. Wild-type eye, adult female.

Fig. 9. Mutant pink-eye (*p*), adult male.*

Fig. 10. Mutant pink-eye (*p*), adult female.*

Fig. 11. Mutant white-eye (*w*), adult male.

Fig. 12. Mutant white-eye (*w*), adult female.

* Differences between the right and left eye colour are due to illumination during photography.

ments were carried out with each strain, and since similar results were obtained with each only the Mak × Somalia cross will be reported here.

A number of reciprocal crosses were made between the Mak diamond and Somalia (*c*) strains. In each cross only the female larvae were taken into consideration owing to the poor penetrance of this character in male larvae. In all cases the F_1 females exhibited the diamond character. Some of the hybrids were inbred to obtain the F_2 generation while others were backcrossed to the Somalia strain. In the case of the inbred there was a close correspondence with the 3:1 distribution of diamond and no diamond females. The backcross progeny segregated in a 1:1 distribution with and without the character, as would be expected if a single gene were involved. A number of these backcrosses were continued for six generations, and in each there was a 1:1 distribution of female larvae with and without the diamond in the thorax. This result was consistent regardless of whether the F_1 male or female was used to introduce the character into the backcross. The results of these experiments seem to indicate the diamond character to be inherited by a single, autosomal, dominant gene. However, it is not felt that the results obtained are sufficient to indicate conclusively monofactorial inheritance and further studies are being made.

(iii) *Red stripe*

This character is similar to the black diamond in that it is best expressed in the female larvae. It appears as a bright red stripe in the mid-dorsal line of the abdomen which broadens out and extends three-quarters of the way along the larval thorax (Plate I, Fig. 7*b*). The red pigment which forms the stripe is most dense in the anterior four or five abdominal segments and least in the posterior segments. It persists into the pupal (Plate I, Fig. 8*b*) and early adult stages. In the adult the stripe can be seen for a short time dorsally in the anterior segments of the abdomen. On the whole, the stripe is usually very faint in males and can only be detected in the larvae.

Originally the red stripe was isolated from the Somalia colony but later was isolated from the Bobo colony as well. Both of these species B group strains originated from a few females with the character and after selection bred true for a number of generations. At present, not enough work has been done to show the type of inheritance involved but it appears to be a polygenic system. Though preliminary results indicated a single dominant gene it was later recorded that when any two strains of freshwater *An. gambiae* were crossed, regardless of species group, the F_1 females had red stripes.

(iv) *Pink eye* (p)

This mutation is visible in the larvae, pupae, and adults. It was isolated from the Lagos colony when pale larvae with large white collars were detected. In some of these larvae the white pigment extended in a broad white stripe down the dorsal abdomen. A detailed examination revealed the larvae, which were pale yellow in colour, to have pale pink eyes (Plate II, Figs. 3*a*, 4). This is in contrast to the orange-

brown eyes of wild-type larvae (Plate II, Fig. 5*a*, *b*) and early pupae which darken to the green colour of the adult eye (Plate II, Figs. 7–8). At emergence mutant males and females have eyes which are almost colourless. The slight pink tinge which is present in both adults is more pronounced in the females. This slight difference persists for a few days after emergence, but by the time the adults are 6 or 7 days old the eyes are an orange-pink of equal intensity in both sexes (Plate II, Figs. 9–10). In addition to the marked differences in eye colour, the mutant and wild-type larvae differ in their body colour. The normal larvae have granules of pigment in the parietal layer beneath the cuticular epidermis which give it a light brown hue (Plate I, Figs. 1, 3). These granules are not present in the pink-eye larvae, which are pale yellow (Plate II, Fig. 3).

The male and female pink-eye mutants were crossed to wild-type individuals in order to ascertain the mode of inheritance of the mutant phenotype. A portion of the F_1 generation was inbred to obtain the F_2 progeny while others were backcrossed. In each backcross the F_1 females were mated with the parental male type. The progeny were separated into the two phenotypes in the larval stage and then allowed to complete their development. In the adult stage the number of males and females in the two phenotypes was recorded for each single pair. The results obtained (Table 2) indicate the pink-eye (*p*) mutation to be sex-linked; when the female parent introduced the mutant character (mating A) into the cross all of the F_1 males were pink-eyed and the females normal. When inbred to the F_2 generation the segregation of pink and normal eye resulted in the expected 1 : 1 : 1 : 1 ratio for a single sex-linked gene. The reciprocal cross (mating B) showed an F_1 phenotype in which all of the progeny were normal-eyed. The F_2 generation corresponded very closely with the expected 3 : 1 ratio of normal and mutant phenotypes. Backcrosses of the F_1 females from crossed A and B to the parental male type gave the expected segregation for a sex-linked recessive gene. All of the segregation data were in good agreement with the hypothesis and there was no significant heterogeneity between parallel pair matings.

Table 2. *Segregation of pink eye* (p)

	Parents (Po)		F_2 segregations			
Mating	♂	♀	+♂	+♀	Pink ♂	Pink ♀
A (10 pairs)	Wild strain × Pink		280	263	272	272
B (10 pairs)	Pink	× Wild strain	460	967	473	0

F_1's from mating A were all pink males and + females, from mating B were all +.

In all of the crosses and backcrosses the pink-eye (*p*) mutant follows a crisscross path of inheritance. The male transmitted his mutant gene via his female progeny to her male progeny, never to or through his male offspring. The mutant character thus alternated from one sex to the other in its passage from generation to generation, which is of course the mode of transmission of the X-chromosone.

(v) *White eye* (w)

This mutant was discovered in the species A Sokoto colony after it had been selected for three generations with DDT. About forty white-eyed males were found amongst the offspring of the selected stock. These mutants were crossed with normal female offspring, and then, by inbreeding, white-eyed males and females were obtained. At first it was necessary to mate the white-eyed males to females by induced copulation techniques; however, as the number of white-eye adults in the cage increased, the strain became self-perpetuating. The *white-eye* gene controls the production of pigment in the eyes of the larva, pupa and adult. It manifests itself by the complete lack of pigment in the larval and developing imago eyes (Plate II, Fig. 1*a*). This condition persists through the pupal (Plate II, Fig. 2) and adult stages (Plate II, Figs. 11, 12) where the eyes are a light cream colour. When cleared in xylol the mutant eyes become translucent. The complete lack of eye pigment makes the mutant readily distinguishable from the wild-type larva, pupa, and adult (Plate II, Figs. 5*a-b*, 6, 7, 8).

The fact that the original mutants isolated were all males, coupled with the results from mating them to normal females, suggested that the mutation was sex-linked. Reciprocal crosses and backcrosses between normal and white-eyed individuals were carried out to confirm this hypothesis. The segregation of the mutant gene in the various crosses is given in Table 3. Only the totals from the single-pair crosses are recorded here; however, the data show that the results obtained, as in the case of the pink-eye gene, depended on the sex of the parent who introduced the mutant into the cross.

Table 3. *Segregation of white eye* (w)

	Parents (Po)		F_2 and backgross (BC) segregations					
Mating	♂	♀		+♂	+♀	White ♂	White ♀	χ_1^2
A (10 pairs)	+ strain × White		F_2	564	622	523	559	4·75
			BC_1	245	465	210	0	2·31
B (10 pairs)	White × + strain		F_2	470	902	398	0	5·95
			BC_2	149	167	130	135	4·49

The F_1's from mating A were all white-eyed males and + females, from mating B were all +. BC_1 and BC_2 were backcrosses of (+)F_1 females to white and + males respectively. The chi-square values for the F_2 of mating A and backcross segregations all show slight deficiencies of white phenotypes from the expected 1 : 1 or 1 : 3 ratio. Mating B, on the other hand, shows a significant deficiency of the mutant phenotypes. There was no significant heterogeneity between replicate matings. Subsequent experiments with the white-eye mutant showed that the earlier the phenotypes were segregated, i.e. in the larvae, the closer the results were to the expected. Though these experiments indicated a mortality in both the white and

+ phenotypes it was much greater in the mutant. This would indicate the deficiencies in the mutant types to be due to their being less viable in the rearing conditions employed. This mortality, though greater in mutant males, did not result in a significant departure from the expected 1 : 1 sex ratio. Thus, although the χ^2 were calculated assuming a 1 : 1 segregation of the sexes they were not biased due to a significant departure from the expected ratio. These results fit the hypothesis and it is concluded that the white phenotype is inherited as a single, recessive, sex-linked gene.

During the course of studying the mutant it was discovered that the white-eye gene has pleiotropic effects on other parts of the larva, pupa and adult. With the exception of melanin in the head capsule, spiracles, and on the base and tips of the hairs, there is no pigment in the body of white-eyed larvae (Plate II, Fig. 1*b*). Mutant larvae are pale yellow and never show white collars, black diamonds or red stripes. This is in contrast with the pink-eye mutation, which does exhibit these characters. However, these genes are similar in that the granules of pigment in the parietal layer beneath the cuticle lacking in pink-eye larvae are also lacking in the white eye. This results in the white-eye larvae being pale cream in colour in contrast with the mottled light brown colour of the wild-type. In the adults the gene for white eye exerted its multiple effects on the testes sheath and accessory glands of the male, and on fecundity in the female. Normal-eyed adult males have yellow accessory glands and produce a like-coloured mating plug (Mason, 1964). In white-eyed males the accessory glands are white and consequently the mating plug is a milky white colour. Similarly the testes sheath, normally pigmented brown, is colourless in the white-eye mutant. The mutant female is so affected by the gene that she produces about one-third fewer eggs than is normal. However, from these the normal percentage, about 90%, become adults.

Another white-eye gene has been isolated in the Kano strain. This gene, though from the *An. gambiae* species B group (Davidson & Jackson, 1962), has almost exactly the same characteristics as those already mentioned for the gene in the species A strain. The only marked difference in the strains is that the female in this strain produces a normal number of eggs, about 150, of which only 50% reach adulthood. In both of these strains the white-eye gene also shortens longevity.

4. EPISTATIC EFFECT OF THE WHITE-EYE GENE

It was noted that among the pleiotropic effects produced by the white-eye mutation was the lack of body pigment in the mutant larvae. It was further recorded that when white-eyed (w) collarless females were crossed to collared (c^+) normal-eyed males (w^+) the resulting F_1 larvae were divisible into two groups, with and without collars. This division conformed to the presence or absence of the white-eye phenotype. Those having white eyes did not have a collar while the normal-eyed larvae expressed the wild-type collar (c^+) gene. Since the collared phenotype is dominant and fully penetrant we would expect all of the F_1 generation to exhibit a white collar. This complete lack of body pigment in the larvae was also

revealed in the white-eyed progeny resulting from hybridizing white-eyed females with males showing black diamonds in the larval stage. The resulting white-eyed larvae, though possessing the potential for white collars (c^+) (Plate I, Figs. 1–2) and diamonds (Plate I, Figs. 5–6), never expressed these characters. At the same time, in all these hybrids, in which we would expect a red stripe, this character was only present in normal-eyed individuals. These results led to the theory that the gene for white eye (*w*) is epistatic to the genes for collar and diamond and the character red stripe. A series of crosses, between white-eyed adults and normal-eyed adults with or without selected characters, was made to test this theory. The parental females were white-eyed and in the larval stage did not have collars, diamonds or red stripes. The males were normal-eyed, from a strain which had been selected for collarless, diamond-thorax larvae. The result of crossing these parental types is shown in Table 4. The F_1 males were white-eyed and collarless while the

Table 4. *Epistatic eflects of the gene for white eyes*

	♂♂	♀♀
Parents:	Normal eye, collarless ×	White eye, collarless
phenotype	diamond	no diamond
	$\left(\frac{w^+}{o}, \frac{c}{c}, \frac{Di}{Di}\right)$	$\left(\frac{w}{w}, \frac{c^+}{c^+}, \frac{Di^+}{Di^+}\right)$

F_1 ♂♂ all white-eyed collarless

$$\left(\frac{w}{o}, \frac{c^+}{c}, \frac{Di^+}{Di}\right)$$

♀♀ all normal-eyed collared, with diamond and red stripe

$$\left(\frac{w^+}{w}, \frac{c^+}{c}, \frac{Di^+}{Di}\right)$$

F_2 Phenotypes (10 pair matings)

White eye	Normal eye			
	Collar		Collarless	
Collarless no diamond	Diamond	No diamond	Diamond	No diamond
515	192	193	76	61

females were normal-eyed, collared, and possessed a diamond and red stripe. The F_2 progeny, ignoring the red stripe due to its uncertain inheritance, showed five segregating larval phenotypes (Table 4) as follows: white eye pigment free, normal eye-collar-diamond, normal eye-collar-no diamond, normal eye collarless-diamond, normal eye-collarless-no diamond. Since the diamond gene as a rule does not penetrate well, if at all, in male larvae, the segregation of this gene did not fit that expected. Limiting the segregation ratio to that between the characters collarless and white eye, the expected F_2 phenotypic ratios would be: 8 white eye-pigment

free, 6 normal eye-collared, and 2 normal eye-collarless larvae. The observed ratios for these two characters were 7·94-5·94-2·12. The segregation data were in good agreement with the expected and there was no significant heterogeneity between parallel pair matings.

Since the normal-eyed strain used in the cross was selected for the recessive gene collarless the presence of collared progeny in the F_1 showed that the *w* strain is c^+/c^+ and white eye is epistatic to the collar gene. The normal segregation in the F_2 without epistasis would be four groups, 3 white-eyed collared, 1 white-eyed collarless, 3 normal-eyed collared, and 1 normal-eye collarless. These were modified in the experiment due to the inability to distinguish between the two white-eyed phenotypes and became 4-3-1. Due to the very poor penetrance of the diamond character in male larvae the segregation ratios did not fit the expected for this character. The presence of diamond and red-stripe females in the F_1 and F_2 but not white-eyed diamond, red stripe showed that *w* is epistatic to diamond and red stripe. It follows from these facts that collar and diamond will only segregate in the F_2 phenotypes which are not homozygous *w*. Thus the white-eye gene acts as a dominant over the genes for collar and diamond and the character red stripe.

5. DISCUSSION

The search for the presence of mutants in the cryptic species A and B of the *An. gambiae* complex has given ample evidence of the genetic plasticity of the strains studied. Since the search was directed towards detecting conspicuous morphological marker genes the mutants listed are only a few of the variants discovered.

In 1956 Davidson demonstrated that resistance to the insectide dieldrin in *An. gambiae* species B was due to a single semi-dominant gene. Following this discovery the genetic basis for resistance to a number of insecticides has been recorded in many species. However, until recently there has been a conspicuous absence of morphological mutants recorded in *Anopheles* species (Davidson & Mason, 1963). This absence of morphological gene descriptions in the literature, in view of all the work carried out on Anopheles mosquitoes, was possibly due to the lack of genetic orientation of the research, or the inability to colonize the strains and mutants. These difficulties have been overcome and methods for colonizing strains are described. The development of induced mating techniques has been a great help, not only for colonizing strains but for studying mutants. The mutants reported here all affect phenotypic changes in the larval and adult pigment. Though the pigments for collar, diamond and red stripe are all located in the parietal layer beneath the larval cuticular epidermis they can occur separately or in various combinations. When all three are present there is a stratification from red to white to black. The pink-eye mutant larvae, unlike the white eye, can occur with any one or combination of these three pigment phenotypes. The lack of expression of these pigments in the white-eye mutant, as has been shown, is due to epistasis. Though nothing is known about the type of pigment present in these phenotypic characters consider-

able information can be extrapolated from the genetic studies on them. Due to the epistatic effect of the white-eye gene all of the pigment genes are unable to express themselves. This includes pigments in the testes sheath, accessory glands and the larval body in general. From these observations it can be considered that all of these pigments require a metabolic process or substance in common for their synthesis. It is, therefore, apparent that the white-eye mutant lacks this substance and no pigment can be formed. Further, the fact that each pigment can occur independently of the others suggests that they each have a different metabolic pathway for their synthesis. The interactions of these genes indicate, and are to be used to show even more effectively, that gene differences lead to certain chemical alterations which result in phenotypic changes.

Of the mutants revealed in this study the most interesting and perhaps the most important are the sex-linked. The white-eye mutation in both *An. gambiae* species A and B and the pink-eye mutation in species A, are the first sex-linked genes to be isolated in any *Anopheline* species. Prior to their isolation all inferences on sex-linkage were based on cytological observations. Most *Anopheles* studied have showed a visible difference in the size of the sex chromosomes (Kitzmiller, 1963); the male in all cases being the heteromorphic sex. The genetic study of the white- and pink-eye genes has confirmed that in *An. gambiae* species A and B, *An. pharoensis* (Mason & Davidson, 1966), and probably in all anophelines with heteromorphic sex chromosomes, there is a *Drosophila* type sex-linkage. This is in contrast with the type of sex-linkage in *Culex molestus* reported by Gilchrist & Haldane (1946, 1947). In the early report they described a mutant sex-linked gene in *C. molestus* affecting eye colour. The later data obtained from crossing white and normal-eyed mosquitoes showed that there were two types of heterozygous white-eye male depending on which parent had been the mutant type. When F_1 males, from a cross between a white-eyed female and a normal male *C. molestus*, were crossed to heterozygous or homozygous recessive females, the resulting white-eyed progeny were almost all female. Conversely, when the F_1 male, resulting from a cross between a white-eyed male and a normal female, was crossed to the heterozygous or homozygous recessive female, almost all the segregating white-eyed individuals were males. In *Anopheles* as has been shown in the present study there is one type of male. The results obtained by Gilchrist & Haldane (1947) led them to the hypothesis that sex in *C. molestus* was determined by a single dominant gene which they called '*M*'. Maleness was assumed to be heterozygous dominant, *Mm*, and females homozygous recessive *mm*. This work has been further substantiated in *C. pipiens* by Laven (1957) and Wild (1963). Another example of the *Mm* mechanism of sex-determination has been demonstrated by McClelland (1966) in *Aedes aegypti*. The morphology of the mitotic chromosomes in *C. pipiens* and *Ae. aegypti* are similar in that there are two large and one small pair of metacentric chromosomes in both sexes (Grell, 1946; Rai & Craig, 1961). The fact that a difference cannot be seen in the smallest pair of metacentric chromosomes tends to indicate agreement with the hypothesis for sex-determination in the *Culicini* put forward by Gilchrist & Haldane (1947). At present very little is known about this subject in *An. gambiae* and *Anopheles* in general.

In most *Anopheles* studied there is a difference in the sex chromosomes of males and females (Kitzmiller, 1963). This heteromorphic state is present in *An. gambiae* (Mason, 1964) and corresponds with the genetic results obtained from studying sex-linked genes. What bearing this has on sex-determination in this species is not known, but it would appear to be different from that in *C. pipiens* and *Ae. aegypti*. This subject offers very interesting possibilities for future research.

SUMMARY

1. A number of mutants affecting changes in the pigment of larval and adult *An. gambiae* are reported.

2. Two other pigment characters are also described. One of these, diamond, is probably inherited as a single factor.

3. Two of the mutants are sex-linked and the experimental results show *An. gambiae* to have a *Drosophila* form of sex-linkage.

4. The sex-linked white-eye mutant is shown to be epistatic to the gene for collar and the characters diamond and red stripe.

5. Attention is drawn to the difference in sex-linkage between *An. gambiae*, and *C. pipiens* and *Ae. aegypti* which may indicate different methods of sex-determination.

I would like to express my appreciation to Professor G. Macdonald for providing space and arranging for grants. Great appreciation and thanks are also expressed to Dr G. Davidson for help and advice. Thanks are also due to Miss J. Chalkley and Mrs C. Routes for their efforts in carrying out the project.

REFERENCES

DAVIDSON, G. (1956). Insecticide resistance in *Anopheles gambiae* Giles: a case of simple Mendelian inheritance. *Nature, Lond.* **178**, 863–864.

DAVIDSON, G. & JACKSON, C. E. (1962). Incipient speciation in *Anopheles gambiae* Giles. *Bull. Wld Hlth Org.* **27**, 303–305.

DAVIDSON, G. & MASON, G. F. (1963). Genetics of mosquitoes. *A. Rev. Ent.* **8**, 177–196.

GILCHRIST, B. M. & HALDANE, J. B. S. (1946). Sex linkage in *Culex molestus*. *Experientia*, **2**, 372.

GILCHRIST, B. M. & HALDANE, J. B. S. (1947). Sex linkage and sex determination in a mosquito, *Culex molestus*. (Univ. Coll) *Hereditas*, **33**, 175–190.

GRELL, S. M. (1946). Cytological studies on *Culex* I. Somatic reduction divisions. *Genetics*, **31**, 77–94.

KITZMILLER, J. B. (1963). Mosquito cytogenetics. A review of the literature, 1953–62. *Bull. Wld Hlth Org.* **29** (3), 345–355.

LAVEN, H. (1957). Vererbung durch Kerngene und das Problem der Ausserkaryotischen Vererbung bei *Culex pipiens*. *Z. indukt. Abstamm.- u. VererbLehre* **88**, 443–477.

MASON, G. F. (1964). Cytogenetics and Genetics of Strains in the *Anopheles gambiae* Complex. Ph.D. Thesis, University of London.

MASON, G. F. & DAVIDSON, G. (1966). Morphological mutants in anopheline mosquitoes. Laboratory meeting. *Trans. R. Soc. trop. Med. Hyg.* **60** (1), 20.

MCCLELLAND, G. A. H. (1966). Sex linkage at two loci affecting eye pigment in the mosquito *Aedes aegypti* (Diptera: Culcidae). *Can. J. Genet. Cytol.* **7** (2), 192–198.

McDaniel, J. M. & Horsfall, W. R. (1957). Induced copulation of Aedine mosquitoes. *Science, N.Y.* **125,** 745.

Rai, K. S. & Craig, G. B. (1961). A study of the karyotypes of some mosquitoes. *Genetics,* **46,** 891.

Wild, A. (1963). A red eye colour mutation in *Culex pipiens* after X-irradiation. *Nature, Lond.* **200,** 917–918.