INHERITANCE AND MAPPING OF HEXOKINASE-1 AND PHOSPHOGLUCOMUTASE IN *ANOPHELES ALBIMANUS* WIEDEMANN[1]

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ABSTRACT. Electrophoretic techniques were used to characterize hexokinase (*Hk*) and phosphoglucomutase (*Pgm*) in the anopheline mosquito, *Anopheles albimanus* Wiedemann. Two zones of hexokinase activity were detected, and inheritance studies were completed for *Hk-1*, the fastest migrating locus in zone 1. *Hk-1* is tightly linked to *reduced palmate* on the left arm of chromosome 3. There was a single, discernible locus for *Pgm* and a total of 5 alleles was detected in laboratory stocks. Genetic crosses were conducted with 3 of these alleles, and the results indicated a loose linkage of these codominant alleles to *green larva (gl)* on chromosome 2.

During recent years, studies have been conducted on the cytogenetics and genetics of *Anopheles albimanus*, the primary vector of human malaria in Central America. These studies have been productive, and significant progress includes mapping of morphological mutants (Benedict et al. 1979), linkage group-chromosome correlation (Rabbani and Seawright, 1976), induction and characterization of chromosome aberrations (Rabbani and Kitzmiller, 1972, 1975, Rabbani et al. 1977), and synthesis of a genetic sexing system for the preferential elimination of females (Kaiser et al. 1978). The genetic sexing strain, MACHO, was implemented in a mass production facility in El Salvador during the conduct of a large pilot study on the release of sterile males as a means of controlling this species (Bailey et al. 1980). A list of the mutant markers available in *An. albimanus* was given by Narang et al. (1981).

With this background, enough basic information is now available for the initiation of studies to develop genetic control mechanisms. The proposed use of chromosome aberrations for the control of insect pests was summarized succinctly by Whitten and Foster (1975).

We are currently working on the induction and characterization of translocations, inversions, and compound autosomes. After characterization, aberrations with the best potential for control will be field tested to determine their suitability for inclusion in autocidal control programs. Since any autocidal program involving a heritable aberration will require mass rearing and release, reliable quality control techniques are needed to monitor the mass production process and the progress of the aberration in competition with the native chromosome type.

After release, the fate of an aberration can be monitored either by examination of the karyotype of field-collected specimens or by the use of mutant markers. Most morphological mutants are not suitable for this purpose because they are usually detrimental and suffer a severe loss in fitness compared to wild type. Also, when recessive traits occur in heterozygous condition, it requires laboratory matings and oviposition to reveal their presence. In addition, it is not al-

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ways possible to obtain eggs from wild-caught females. Therefore, examination of cytological preparations will be of utmost importance in tracking the aberration. However, for the genetic monitoring of mass production and cryptic variability in the field population during the course of a release program, the most readily available method is the use of allozymes. The codominant nature of allozymes allows the visualization of the genotype of a specimen after electrophoresis. The isoenzymes of every insect sampled provide data on its genotype. The availability of a large number of naturally-occurring allozyme variants means that each linkage group can be marked at several locations. Thus, an aberration, e.g. any inversion or compound chromosome, can be tagged and followed in a mixed population.

We have undertaken genetic studies on several enzyme systems in *An. albimanus* to identify and map suitable allozyme markers. In this present paper, we report our results on hexokinase (*Hk*) and phosphoglucomutase (*Pgm*), including the inheritance pattern, linkage group analysis, and gene frequency of the alleles in 4 laboratory stocks.

MATERIALS AND METHODS.

Two laboratory strains of *An. albimanus* were used to isolate homozygous stocks for alleles of hexokinase (*Hk*) and phosphoglucomutase (*Pgm*): (1) *gl rp st*—This strain is homozygous for the recessive traits, *green larva (gl)* on chromosome 2 (Seawright et al. 1979a) and *reduced palmate (rp)* (Seawright et al. 1979b), and *nonstripe (st)* (Rabbani and Seawright 1976) on chromosome 3. All 3 markers are expressed during the 4th larval stage, and *st* and *gl* are also visible in the pupae. (2) SANTA TECLA—This strain was originally collected in El Salvador and contains *gl* and *st*, as well as their dominant alleles st^+ *(stripe)* and gl^+ *(tan)*. A strain homozygous for gl^+, st^+, and rp^+ and another strain homozygous for gl^+, *st*, rp^+ were isolated. The scheme used to isolate stocks homozygous for the alleles of *Hk-1* and *Pgm* was the same as reported by Narang et al. (1980a) and involved a combination of single-pair and mass mating procedures.

The various laboratory stocks that were analyzed for gene frequency distributions are listed in Table 1. Electrophoretic and zymogram techniques employed in these studies were reported by Steiner and Joslyn (1979). For hexokinase, their lithium and CA-7 buffer systems proved better than CA-8 for resolution in *An. albimanus* and were employed for analyzing crosses. The CA-7 buffer system was employed for the studies of *Pgm*. Tris borate-EDTA buffer (Narang and Kitzmiller 1972) was also used for hexokinase in vertical acrylamide electrophoresis for comparing zymogram results.

RESULTS

In Fig. 1, a summary is presented of the variability in activity of hexokinase obtained during the analysis of numerous zymograms. There were 2 zones migrating toward the anode, but hexokinase activity, as expressed by the banding pat-

Table 1. Distribution of alleles of *Hk-1* and *Pgm* in laboratory stocks of *An. albimanus*.

	Frequency of allele						
	Hk-1			*Pgm*			
Stock	n	S	F	n	S	I	F
gl rp st	85	1.0	—	73	0.71	0.17	0.12
CAMPO	30	0.38	0.62	30	0.02	0.73	0.25
SANTA TECLA	48	0.43	0.57	75	0.18	0.73	0.09
APASTEPEQUE	27	0.46	0.54	34	0.50	0.12	0.38

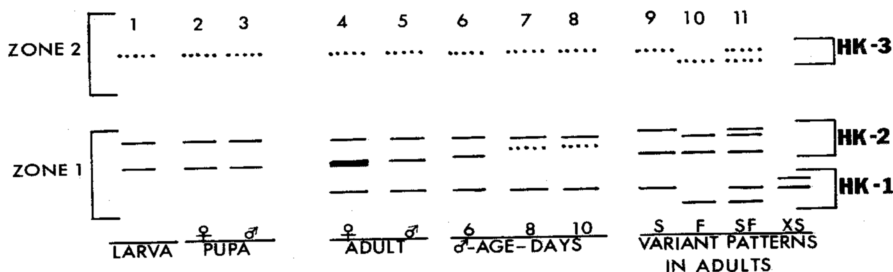

Fig. 1. A schematic drawing of hexokinase phenotypes revealed during developmental stages of *An. albimanus*. At the Hk-1 locus more frequent phenotypes are S, F, and SF. XS is a very rare electromorph and has not been investigated by genetic analysis.

terns, varied somewhat, depending on the life stage (larva, pupa, adult) and age of the adult. The *Hk-1* locus was absent in larvae and pupae, but showed strong activity in the adult. The zymograms of parents and their progeny on the same gel clearly showed that the zone I bands are under the control of 2 loci, *Hk-1* and *Hk-2*. Individuals homozygous at the *Hk-1* locus showed a single band of either slow (S) or fast (F) type, and the heterozygotes showed the expected 2 bands of the monomeric hexokinase. Most of the laboratory strains are polymorphic for *Hk-1* except the *gl rp st* which is fixed for S allele (Table 1). A 3rd allele, X, with lesser mobility, was rarely observed and merits further study. In addition to the S and F alleles of *Hk-1*, there were 2 other bands commonly observed in zone-I. This two-banded pattern is more than likely under the control of a single locus, *Hk-2*. Both bands were distinctly visible in larvae and pupae, and the most anodic band stained intensely in newly emerged adult females, but with increasing age this band became less visible in males. More work is required before the effects of age, sex, and life stage on expression of the 2 bands can be adequately assessed. A very low level of variability was noted for the two-banded *Hk-2*. Similarly, one-band or two-band electromorphs in zone II are expressions of the alleles of *Hk-3*.

Thus far, we have conducted inheritance and mapping studies for *Hk-1*, and the crosses involving this locus are summarized in Tables 2 and 3. *Hk-1* assorted independently from *green larva (gl)* (Table 2) and showed tight linkage to *rp* on chromosome 3. The linkage estimates for *st* and *rp* are similar to the previous estimate of 17.1 map units that was reported by Seawright et al. (1979b). Work of Narang et al. (1981) shows that 2 esterase loci (*E-4* and *E-8*) are located on the left arm of chromosome 3. Combining their estimates with the data in Tables 2 and 3, the following gene sequence was obtained for chromosome 3.

E-8 . . . . . *E-4* . . . . *Hk-1* . . . *rp* . . . . . . *st*

An electropherogram for *Pgm* in adult mosquitoes is shown in Figure 2. For the alleles we studied, a total of 6 phenotypes were observed in laboratory populations, and the banding pattern of parents and their progeny on the same gel indicated that they resulted from segregation of 3 alleles at a single, autosomal locus. Laboratory strains differ in allelic frequencies (Table 1). In addition to the locus in the major zone, a 2nd (more anodic) zone of weak activity was noted in gels incubated for more than 3 hours. No polymorphism was detected in this 2nd zone. When the lithium buffer system (Steiner and Joslyn 1979) was used, this zone of weak activity migrated cathodic to the 1st zone.

Table 2. Summary of crosses with Hk-1 showing linkage with the chromosome 3 markers, *stripe* (st^+) and *reduced palmate* (*rp*). Map distances were: *st – Hk-1* = 19.21±1.85; and *rp – Hk-1* = 1.49±0.61. No linkage was noted between Hk-1 and *green larva* (*gl*) on chromosome 2.

Cross ♀ × ♂	No. of families	Phenotype of progeny: Parental	Phenotype of progeny: Recombinant	Total	Linkage $\overline{X}^2$	Percent recombinants	
F_1 ($st^+Hk\text{-}1^F$ × $st\ Hk\text{-}1^S$) × $st\ Hk\text{-}1^S$	4	197	47	244	92.21*	19.26±2.51	$\overline{X}$ = 19.21 ± 1.85
$st\ Hk\text{-}1^S$ × F_1 ($st^+Hk\text{-}1^F$ × $st\ Hk\text{-}1^S$)	1	76	21	97	31.18*	21.65±4.18	
$st\ Hk\text{-}1^S$ × F_1 ($st\ Hk\text{-}1^S$ × $st^+Hk\text{-}1^F$)	1	93	19	112	48.89*	16.96±3.55	
F_1 ($rp^+\ Hk\text{-}1^F$ × $rp\ Hk\text{-}1^S$) × $rp\ Hk\text{-}1^S$)	2	138	2	140	132.11*	1.43±1.00	$\overline{X}$ = 1.49 ± 0.61
$rp\ Hk\text{-}1^S$ × F_1 ($rp^+\ Hk\text{-}1^F$ × $rp\ Hk\text{-}1^S$)	2	145	4	149	133.43*	2.68±1.32	
$rp\ Hk\text{-}1^S$ × F_1 ($rp\ Hk\text{-}1^S$ × $rp^+Hk\text{-}1^F$)	1	112	0	112	—	0.00	
F_1 ($gl^+\ Hk\text{-}1^F$ × $gl\ Hk\text{-}1^S$) × $gl\ Hk\text{-}1^S$)	6	153	178	331	1.88	no linkage	
$gl\ Hk\text{-}1^S$ × F_1 ($gl^+\ Hk\text{-}1^F$ × $gl\ Hk\text{-}1^S$)	2	71	69	140	0.03	no linkage	

* Linkage $\times^2$ highly significant.

Table 3. Three-point test crosses showing the linkage relationship between *non-stripe* (*st*), *reduced palmate* (*rp*) and *hexokinase-1* (*Hk-1*). Linkage distances were calculated; *st-rp* = 17.38±1.85; *st-Hk-1* = 18.33±1.89; *rp-Hk-1* = 0.95±0.47.

Crosses ♀ × ♂	No. families	$st^+\ rp^+$ S/S	$st^+\ rp^+$ S/F	$st^+\ rp$ S/S	$st^+\ rp$ S/F	$st\ rp^+$ S/S	$st\ rp^+$ S/F	$st\ rp$ S/S	$st\ rp$ S/F	Total
$rp\ st\ Hk\text{-}1^S$ × F_1 ($rp^+st^+\ Hk\text{-}1^F$ × $rp\ st\ Hk\text{-}1^S$)	1	1	36	8	0	0	5	29	0	79
F_1 ($rp^+\ st^+\ Hk\text{-}1^F$ × $rp\ st\ Hk\text{-}1^S$) × $rp\ st\ Hk\text{-}1^S$	2	1	46	9	0	0	14	61	0	131
$rp\ st\ Hk\text{-}1^S$ × F_1 ($rp\ st\ Hk\text{-}1^S$ × $rp^+\ st^+\ Hk\text{-}1^F$)	1	0	54	12	0	0	7	39	0	112
F_1 ($rp\ st\ Hk\text{-}1^S$ × $rp^+\ st^+\ Hk\text{-}1^F$) × $rp\ st\ Hk\text{-}1^S$	1	2	37	12	0	0	6	41	0	98

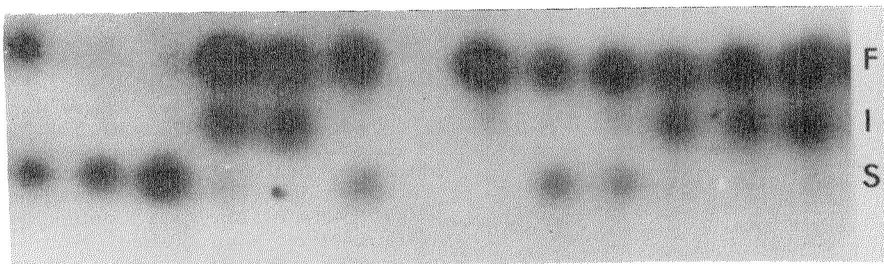

Fig. 2. A photograph of a *Pgm* zymogram prepared from adult mosquito homogenates showing at *Pgm* locus. The secondary *Pgm* band of variable activity is almost always present both in homozygotes and heterozygotes. (Faint band in 4th sample from left).

In test crosses, *Pgm* assorted independently of *rp* and *st* (on chromosome 3), and a loose linkage was detected between *Pgm* and *gl* (Table 4). A significant χ^2 value was not observed when crosses involving *gl* and *Pgm* were analyzed separately because the sample size was too small to accurately detect a loose linkage. However, for the combined data, a significant χ^2 (= 12.51, P<.01) was calculated, and the maximum likelihood estimate of map distance was 44.56±1.53, which is undoubtedly an underestimate of the true distance because of double crossingover. The train *gl* is located near the free end of 2R (Seawright et al. 1979a) at about 30 map units from the centromere; therefore, *Pgm* is tentatively assigned to the left arm of chromosome 2.

DISCUSSION

Genetic studies on hexokinases of anopheline species were very limited until recently when Steiner et al. (1979) described polymorphism for several species from Brazil. They noted 3 distinct zones of activity for zymograms prepared from adult mosquitoes. The zone located closest to the anode was designated Hk-F, and their data indicated this zone was under the control of an autosomal local with 2 co-dominant alleles. The heterozygotes at this locus show 2 bands as expected. A middle zone (Hk-M) consisted of 2 bands in each mosquito and was interpreted as a one-locus—two-band system. Homozygotes for the alleles at this locus also showed 2 bands, differing only in 1 of the 2 standard bands, and for heterozygotes there were 3 bands. Their slowest, third zone (Hk-S) consisted of a one-gene—one-band type displaying faint hexokinase activity, and 3 bands observed at this locus indicated 2 codominant alleles. They also stated that Hk-F and Hk-M are not completely independent, a condition referred to as "simultaneous heterozygosity" for these 2 loci. Individual mosquitoes that were heterozygous (three bands) at Hk-M almost always revealed 2 bands for Hk-F. The reverse was not true. This same sort of phenomenon has also been observed in other anophelines (Steiner-personal communication).

Our observations of hexokinase activity in *An. albimanus* paralleled those described by Steiner et al. (1979), with the important exception that no "simultaneous heterozygosity" was seen. The *Hk-1*, *Hk-2*, and *Hk-3* designations which we employed are comparable to Hk-F, Hk-M, and Hk-S. A survey of several laboratory strains revealed polymorphism for the Hk-1 locus, but there was never an indication of any interaction between Hk-1 and Hk-2. The location of Hk-1 on 3L provides a very useful tool for marking chromosome aberration stocks involving chromosome 3.

The inheritance pattern and structure

Table 4. Summary of crosses with *Pgm* showing independent assortment from *st* and *rp* chromosome 3 and a loose linkage with *gl* on chromosome 2.

Crosses ♀ × ♂	No. families	Phenotype of progeny: Parental	Recombinant	Total	χ^2 Test for linkage: Separate	Combined
F_1 ($st\ Pgm^S \times st^+\ pgm^F$) × $st\ Pgm^S$	4	192	200	392	0.16	$\chi^2 = 0.083$ (no linkage)
$st\ Pgm^S \times F_1$ ($st\ Pgm^S \times st^+\ Pgm^I$)	2	92	82	174	0.57	
F_1 ($st^+\ Pgm^I \times st\ Pgm^S$) × $st\ Pgm^S$	3	147	151	298	0.05	
$st\ Pgm^S \times F_1$ ($st^+Pgm^I \times st\ Pgm^S$)	1	62	51	113	1.07	
$rp\ Pgm^S \times F_1$ ($rp\ Pgm^S \times rp^+\ Pgm^F$)	2	87	80	167	0.29	$\chi^2 = 0.530$ (no linkage)
F_1 ($rp\ Pgm^S \times rp^+\ Pgm^F$) × $rp\ Pgm^S$	3	141	135	276	0.13	
F_1 ($rp^+\ Pgm^F \times rp\ Pgm^S$) × $rp\ Pgm^S$	1	60	62	122	0.03	
$rp\ Pgm^S \times F_1$ ($rp^+\ Pgm^F \times rp\ Pgm^S$)	1	62	54	116	0.55	
F_1 ($gl\ Pgm^S \times gl^+\ Pgm^F$) × $gl\ Pgm^S$	1	47	37	84	1.19	$\chi^2 = 12.51$ ($P < .01$) Crossingover = 44.56%
$gl\ Pgm^S \times F_1$ ($gl\ Pgm^S \times gl^+Pgm^F$)	3	261	205	466	6.73	
F_1 ($gl^+Pgm^F \times gl\ Pgm^S$) × $gl\ Pgm^S$	4	175	145	320	2.81	
$gl\ Pgm^S \times F_1$ ($gl^+\ Pgm^F \times gl\ Pgm^S$)	2	103	84	187	1.93	

of hexokinase of *An. albimanus* show some similarities as well as differences from similar studies on other mosquito species. Genetic analysis of hexokinase has been reported for *Culex* sp., *Aedes aegypti* (L.), and *Culiseta inornata* (Williston) (Narang et al., unpublished), and populations of diverse origin (Australian, Oriental, and European) of the *Culex pipiens* complex (Miles 1974). For the species studied by Narang et al. (unpublished) electrophoretically detected hexokinase activity was similar to that reported by Steiner et al. (1979) for anophelines of the subgenus, *Nyssorhynchus*. There was one very important difference. The 3 bands (2 bands of Hk-M and 1 band of Hk-F), which are the result of 2 loci in the anophelines and other species, are due to a single locus in the Boston population of *Culex pipiens molestus* L. A homozygote produces a three-band phenotype and heterozygotes always show 6 bands. Work by Miles (1974) suggested that the Hk-F locus is missing in those populations of the *Culex pipiens* complex that he examined. He also showed no difference in expression of hexokinase activity in relation to the stage of development of the mosquitoes. However, in our studies of 11 species of anophelines and *Culex quinquefasciatus* Say from Brazil, the Hk-F zone was consistently absent when larvae and pupae were analyzed. These conflicting results indicate a need for more study on the effect of electrophoretic conditions and buffer systems on measuring hexokinase activity.

Allozymes of *Pgm* of *An. albimanus* have a simple monofactorial inheritance. The structure of this enzyme results in a monomer molecule and thus mosquitoes heterozygous at the *Pgm* locus show 2 bands corresponding to the respective alleles. This 1 gene-one band situation, typical of this species, holds true in 13 other species of neotropical anopheline mosquitoes of the subgenus *Nyssorhynchus* (unpublished data). Though the same is true for most of the palearctic anophelines, a 1 gene-two band, with the 2nd weaker band migrating anodic to the

principal band, has been reported to occur in *An. claviger* (Meigen) and *An. maculipennis* Meigen (Bullini and Coluzzi 1973). A different phenomenon occurs in *Culex p. quinquefasciatus* Say where each allele produces two bands of equal intensity (Cheng and Hacker 1976). It is not clear whether the second band is a primary gene product or represents post-translational modification of the primary genetic product. Further studies are needed on these species to understand better the genetic basis of the one gene-two band electromorph patterns.

Although we isolated stocks homozygous for only 3 alleles to study the inheritance and linkage group relationship of *Pgm* in *An. albimanus,* a total of 5 alleles was detected among various laboratory strains of this species. Of these, the 3 more frequent alleles have been analyzed. The remaining two alleles occur in low frequency. Since single pair mating in this species is not routinely feasible, no detailed genetic analysis was possible on these alleles. The relatively high level of *Pgm* polymorphism in this species is consistent with similar reports on other mosquito species. Among palearctic anophelines of the subgenus *Anopheles,* a total of 6 *Pgm* alleles have been reported in sibling species of the *An. maculipennis* complex, with 4 alleles in *An. plumbeus* Stephens, 3 in the *An. claviger* (Meigen) group, and 2 alleles in a single sample of the Mediterranean species *An. algeriensis* Theobald (Bullini and Coluzzi 1973). Among anophelines of the subgenus *Cellia* a total of 4 *Pgm* alleles was reported in 6 sibling species of the Ethiopian group of the *An. gambiae* Giles complex. Three alleles occur in one population of a Mediterranean species, *An. hispaniola* (Theobald). In *Neocellia* a total of 5 alleles occurs in various geographic strains of *An. stephensi* Liston from Southeast Asia (Bullini et al. 1971 a,c, & 1973 a,c).

Among *Aedes* mosquitoes as many as 7 *Pgm* alleles were found in 19 populations of different geographic origin of *Aedes aegypti,* a mosquito of wide distribution in tropical, subtropical, and temperate zones (Bullini et al. 1970 a, b 1972a, 1973b). A total of 5 alleles occur in 19 natural populations of different geographical origin of 3 sibling species of *Aedes mariae* (Sergent & Sergent) complex (Coluzzi et al. 1971a,b and Coluzzi and Bullini 1971). An analysis of 8 populations of *Culex p. pipiens* and 6 populations of *Culex p. molestus* Forskal from Italy revealed 3 to 6 *Pgm* alleles (Bullini et al. 1971b and 1973c).

References Cited

Bailey, D. L., R. E. Lowe, D. A. Dame and J. A. Seawright. 1980. Mass rearing the genetically altered MACHO strain of *Anopheles albimanus.* Am. J. Trop. Med. Hyg. 29:141–149.

Benedict, M. Q., J. A. Seawright, D. W. Anthony and S. W. Avery. 1979. *Ebony,* a semidominant lethal mutant in the mosquito, *Anopheles albimanus.* Can. J. Genet. Cytol. 21:193–200.

Bullini, L., M. Coluzzi, A. M. Gironi and M. Morellini. 1970a. Phosphoglucomutase polymorphism in *Aedes aegypti.* Parassitologia 12:27–30.

Bullini, L., A. M. Gironi, A. P. Bianchi Bullini and M. Coluzzi. 1970b. Further observations on phosphoglucomutase polymorphism in *Aedes aegypti.* Parassitologia 12:113–117.

Bullini, L., G. Cancrini, A. P. Bianchi Bullini and M. DiDeco. 1971a. Further studies on the phosphoglucomutase gene in *Anopheles stephensi:* evidence for a fourth allele (Diptera:Culicidae). Parassitologia 13:435–438.

Bullini, L., M. Coluzzi, A. P. Bianchi Bullini and B. Gleiner. 1971b. Phosphoglucomutase polymorphism in *Culex pipiens* (Diptera:Culicidae). Parassitologia 13:439–443.

Bullini, L., M. Coluzzi, G. Cancrini and S. Santolamazza. 1971c. Multiple phosphoglucomutase alleles in *Anopheles stephensi.* Heredity 26:475–478.

Bullini, L., A. M. Gironi, A. P. Bianchi Bullini and M. Coluzzi. 1972. Phosphoglucomutase gene in *Aedes aegypti:* A fourth allele and preliminary linkage data. Biochem. Genet. 7:41–44.

Bullini, L. and M. Coluzzi. 1973. Electrophoretic studies on gene-enzyme systems in mosquitoes (Diptera:Culicidae). Parassitologia 15:221–248.

Bullini, L., G. Cancrini. G. Mara, M. DiDeso and B. Bullini. 1973a. Alleli per la fosfoglucomutasi in popolazioni di *Anopheles*

stephensi di diversa orgine geografic. Parassitologia 15:217–220.

Bullini, L., M. Coluzzi, A. P. Bianchi Bullini and G. Cancrini. 1973b. A new phosphoglucomutase (Pgm) allele in *Aedes aegypti* (Diptera:Culicidae). Parassitologia 15:141–144.

Bullini, L., M. Coluzzi, A. P. Bianchi Bullini and L. Renna. 1973c. Stability of frequencies of phosphoglucomutase alleles in *Culex pipiens* breeding in ecologically different environments. Acc. Naz. Lincei Rend. Cl. Sc. Fis. Mat. e. Nat. 53:608–611.

Cheng, M. L. and C. S. Hacker. 1976. Inheritance of 6-phosphogluconate dehydrogenase variants in *Culex pipiens quinquefasciatus* Say. J. Heredity 67:215–219.

Colluzi, M. and L. Bullini. 1971. Enzyme variants as markers in the study of precopulatory isolating mechanisms. Nature: 231:455–456.

Coluzzi, M., L. Bullini and A. P. Bianchi Bullini. 1971a. Phosphoglucomutase (Pgm) allozymes in two forms of the *mariae* complex of the genus *Aedes*. Biochem. Genet. 5:253–255.

Coluzzi, M., L. Bullini and A. P. Bianchi Bullini. 1971b. Phosphoglucomutase polymorphism in *Aedes phoeniciae* Coluzzi and Sabatini of *Ae. mariae* complex (Diptera: Culicidae). Bull. Entomol. Res. 61:327–330.

Kaiser, P. E., J. A. Seawright, D. A. Dame and D. J. Joslyn. 1978. Development of a genetic sexing system in *Anopheles albimanus*. J. Econ. Entomol. 71:766–771.

Miles, S. J. 1974. Biochemical polymorphisms and evolutionary relationships in the *Culex pipiens* complex (Diptera:Culicidae). Ph.D. Dissertation, University of Western Australia, Perth. 115 p.

Narang, S. and J. B. Kitzmiller. 1972. Dehydrogenase polymorphism in *Anopheles punctipennis* (Diptera:Culicidae). Genetics of xanthine and octanal dehydrogenases. Ann. Entomol. Soc. Amer. 65:798–804.

Narang, S., J. A. Seawright and J. B. Kitzmiller. 1981. Linkage relationship and assignment of *Esterase-4* and *Esterase-8* loci to the left arm of chromosome 3 in *Anopheles albimanus*. J. Heredity (In press).

Rabbani, M. G. and J. B. Kitzmiller. 1972. Chromosomal translocations in *Anopheles albimanus* Wiedemann. Mosq. News. 32:421–432.

Rabbani, M. G. and J. B. Kitzmiller. 1975. Studies on X-Ray induced chromosomal translocations in *Anopheles albimanus* I. Chromosomal translocations and genetic control. Amer. J. Trop. Med. Hyg. 24:1019–1026.

Rabbani, M. G. and J. A. Seawright. 1976. Use of Y-autosome translocations in assigning the *stripe* locus to chromosome 3 in the mosquito, *Anopheles albimanus*. Ann. Entomol. Soc. Amer. 69:266–268.

Rabbani, M. G., J. A. Seawright and J. B. Kitzmiller, 1977. Xray induced pericentric inversions in *Anopheles albimanus* Wiedemann. Can. J. Genet. Cytol. 19:67–74.

Seawright, J. A., L. V. Childress and M. Q. Benedict. 1979a. Genetics of *green larva*, a recessive mutant on chromosome 2 in *Anopheles albimanus* Weidemann. Mosq. News 39:55–58.

Seawright, J. A., M. Q. Benedict and S. G. Suguna. 1979b. Reduced palmate, a recessive mutant on chromosome 3 in the mosquito, *Anopheles albimanus*. J. Heredity 70:321–324.

Steiner, W. M. M., S. Narang and J. B. Kitzmiller. 1979. Interaction at two hexokinase loci in neotropical anophelines. Isozyme Bull. 12:66–67.

Steiner, W. M. M. and D. J. Joslyn. 1979. Electrophoretic techniques for the genetic study of mosquitoes. Mosq. News 39:35–54.

Whitten, M. J. and G. G. Foster, 1975. Genetical methods of pest control. Ann. Rev. Entomol. 461–476.