Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1964, 31, 469-473

Applications of Genetic Technology to Mosquito Rearing*

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Genetical considerations are frequently ignored by entomologists engaged in the laboratory rearing of insects for research. Many workers have assumed that strains maintained under long laboratory colonization are therefore fairly homogeneous. This has led them to expect uniform responses within strains and between laboratories. As a consequence, results are variable and significance is much reduced. Little effort has been made to standardize strains for research in insect physiology or toxicology. This disinterest in standardization is in marked contrast to the emphasis placed on pure lines and standard strains by workers in other areas of biology.

The present paper will indicate that laboratory strains of mosquitos are indeed variable. Furthermore, a way of reducing this variability is proposed and a method for improving insects reared for experimental purposes or for autocidal programmes is described. These remarks are a result of experience with *Aedes aegypti*. With modification, they should apply to other mosquitos and, indeed, to other species of insects which can be reared in the laboratory.

GENETIC VARIABILITY

It is a basic tenet of modern biology that organisms differ from one another, both within species and within populations. A large portion of this variability is genetic. Genetic variability confers plasticity, which is beneficial to both species and individuals. The more kinds of genes present in a gene pool, the greater is the possibility for adaptation to diverse environments.

Unfortunately it is difficult to measure the extent of genetic variability in a population. The great majority of recessive genes in a heterozygous population are hidden by their dominant counterparts. Moreover, the genes conferring morphological changes form only a small portion of the total amount of potential variability. Most genes affect characters that are not visible, rather acting on physiological processes. Complex genetic techniques are necessary to measure variability in such genes and these techniques are available in few insects other than *Drosophila*.

Because we may not be able to see gross differences in a group of organisms such as a laboratory colony of mosquitos, we often assume that they are all alike, or nearly so. This is certainly a mistake. Genetic analysis has repeatedly demonstrated the uniqueness of the individual. Practically speaking, every mosquito is different from every other mosquito.

This variability is evident in *Aedes aegypti*. Various genetic differences within and between populations of this species have been described by Mattingly, McClelland, Gillett, Wood and others. Craig, VandeHey & Hickey (1960) showed that *A. aegypti* is characterized by great genetic plasticity. VandeHey (1961) made a quantitative analysis of the frequency of morphological mutants in laboratory strains. By inbreeding for several generations he was able to uncover and score mutants carried in the heterozygous condition. He found 142 mutants of 25 different kinds from 104 mosquitos, giving an average of 1.36 mutants per mosquito. Comparable studies in *Drosophila* have given frequencies of 0.5 to 1.0, yet one thinks of *Drosophila* as showing considerable genetic plasticity.

The results of VandeHey are from laboratory strains. In three colonies of diverse origin he obtained frequencies of 1.58, 1.41 and 1.10 mutations per mosquito. Several of these colonies had been reared in the laboratory for more than 10 years. According to the concepts of all too many investigators, these strains should be highly homogeneous.

* The work of the author, reported in this paper, received support from the following sources: (1) Research Grant No. AI-02753-04, National Institutes of Health, Public Health Service, US Department of Health, Education, and Welfare; (2) Research contract No. DA-18-064-AMC-52 (A), US Army Biological Laboratories; (3) The Radiation Laboratory of the University of Notre Dame, US Atomic Energy Commission Contract No. AT (11-1)-38.

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Craig & Hickey (1961) conducted similar experiments on field-collected *A. aegypti* from Africa. Frequency of mutants per mosquito in feral populations from Nigeria and the Congo was 0.72 and 0.84, while two domesticated populations from Kenya gave 2.96 and 2.72. It is interesting to note that the laboratory populations were intermediate between the two groups of field populations. These data contradict the prevalent notion that laboratory colonization must greatly restrict variability. Work with *Drosophila* bears out the premise that laboratory colonies may be more or less variable than field populations.

What are the sources of genetic variability in laboratory populations? They are the forces responsible for evolution in the field: mutation, selection, isolation, genetic drift. Selection is probably most important because it is operating on all populations at all times. When a strain is colonized by removal from field to laboratory, it undergoes drastic selection and the gene pool changes quickly. However, conditions in the laboratory are never really constant and change continues there. For example, the microbiota in larval rearing media may undergo seasonal changes. In our laboratory, rearing is done under conditions of constant temperature, humidity and lighting, yet the airborne micro-organisms increase greatly during the spring and this increase affects larval growth.

Selective pressures operate in different ways in different laboratories. In a survey of the gene for yellow larvae in 25 laboratory strains of *A. aegypti*, Craig, VandeHey & Hickey (1960) found gene frequencies ranging from 0.1 to 1.0. Geographic location and strain origin had relatively little influence on frequency. Moreover, strains colonized and maintained at one laboratory and then reared elsewhere for several years showed marked changes in gene frequency. They concluded that this gene showed different levels of balanced polymorphism in different laboratories. Adhami (1962) obtained experimental verification of this point. He established population cages having yellow larvae at 10%, 50% and 90% of the population. After several years of continuous rearing, all three cages had about 75% yellow larvae, the equilibrium point for this gene under conditions in his laboratory.

Since strains are constantly changing, it seems unfortunate to name a strain after its laboratory of origin after it has been reared elsewhere. Many workers in the USA use the so-called " Orlando " strain, even though their material was removed from the Orlando laboratory colony 10 or 20 years ago. Their present strain will bear but faint resemblance to the original Orlando strain after that time.

To summarize, laboratory strains are evolving perhaps more rapidly than field populations. Gene frequencies are changing constantly. It is most improbable that any two individuals or any two generations would be identical.

GENETIC STANDARDIZATION

The research worker needs genetically uniform insects. By keeping the genetic constitution constant, one has a better chance to determine the significance of experimental treatment. Moreover, genetic uniformity must be attained before experiments can be repeated, either within or between laboratories. One would expect research papers to pay as much attention to the genetic composition of experimental animals as to method and apparatus. This is certainly not the case in mosquito literature today.

Genetic uniformity can be obtained by inbreeding. The best system for mosquitos should be through single-pair, brother-sister matings for a series of generations. This system reduces the amount of heterozygosity in each generation. After 20 generations of uninterrupted brother × sister matings, 99% of all gene pairs are, theoretically, homozygous. Forty generations are required to reach 99.994%. It is impracticable and probably impossible to reach complete homozygosity in diploid, bisexual organisms. For most practical purposes, however, 99% homozygosity suffices and such lines may be considered as *isogenic*. Among mouse geneticists, the Committee on Standard Genetic Nomenclature for Mice has limited the term " inbred strain " to those families which have been bred only brother × sister and/or parent × offspring for 20 generations. The term " substrain " is applied to a group of inbred animals separated from the parent inbred strain and propagated by inbreeding. The 20-generation limitation is not generally used by other workers.

Inbred strains of *Aedes aegypti* have been available from the University of Notre Dame for several years. Four lines, derived from different strains, are currently maintained (Kenya, Rock, X_2, Congo) and all are at about F_{19} or F_{20}. In each case, inbreeding was most difficult during generations F_4 to F_7. Factors for sterility and reduced vigour were responsible for loss of numerous lines at this time. However, those lines surviving to the F_8 and thereafter were viable and no difficulties were encountered in subsequent generations. It seems that the highly

deleterious genes were weeded out and the lines are now reasonably homozygous.

These isogenic strains of *Aedes aegypti* have been of considerable value in genetical studies on biochemistry, development and disease transmission. However, there are certain disadvantages in using them. Vigour, fecundity and longevity are much reduced. In order to obtain large numbers for experimental purposes it is necessary to mass-cross the inbred progeny. This can be done for several generations without significant loss of homozygosity. However, after five or six generations, new mutants begin to accumulate and the strain begins to resemble a random-breeding, polymorphic laboratory strain. Therefore, the inbreeding programme must be maintained without interruption and expansion through mass crossing must be limited.

A second disadvantage of the inbred strain is in the area of uniformity of response. Workers with many organisms have noted that isogenic strains sometimes show wider variation in response to experimentation than do random-bred strains. It appears that inbreeding may reduce buffering of the environment. Thus, development time, longevity, or response to toxicants may be highly variable among individuals with the same genotype.

THE F_1 HYBRID

There is a simple way of avoiding the disadvantages of inbreeding without sacrificing genetic uniformity. The F_1 hybrid resulting from the cross between two inbred lines provides the best possible experimental animal. F_1 individuals are usually more vigorous, hardy and generally more fit than either parental stock. Indeed, their average fitness often exceeds that in any random-bred strain. Moreover, heterosis ensures uniformity of response to experiment and synchronized growth and development. F_1 organisms are genetically uniform because all individuals in the population have the same haploid set of chromosomes from each inbred parent. The only major disadvantage in such a programme is that the test organisms cannot be used for breeding purposes. Heterosis breaks down after the F_1 generation. The F_2s show great variability and hence are of no value.

Experiments at our laboratory have demonstrated useful levels of heterosis in *Aedes aegypti*. We have crossed inbred lines and measured a number of biological characters. The following characters were improved by heterosis:

1. Larval development time was reduced.
2. Variation in the time of pupation and of adult emergence was reduced.
3. Mortality in the immature stages was reduced.
4. Adult longevity was increased.
5. Egg production was increased.
6. Resistance of eggs to radiation was increased.

In some experiments heterosis decreased larval development time by more than 25%. In reciprocal F_1 hybrids, 96% and 70% had pupated five days after hatching, while the parental stocks required seven to eight days to reach a similar condition. Reduction in mortality in the immature stages was also striking. In one case, 94% and 76% of newly hatched larvae from the parental stocks survived to the adult stage. This may be compared with 100% for both of the reciprocal F_1s.

The advantage of heterosis was most evident under conditions of stress. Adult longevity was measured in individuals from larvae reared at 26°C and 30°C. The F_1 from the lower temperature lived 43% longer than the parent strains. However, the F_1 from larvae reared at 30°C lived 75% longer.

The heterotic improvement was also evident in crosses between random-breeding strains. Crosses between the Rock and Red Eye strains were made in connexion with experiments on the effect of radiation on egg development. Considering the time required to complete pupation, the Rock parent required 28% and the Red Eye required 71% more time than did their F_1 progeny. It is interesting to note that the hybrids were more resistant to radiation than the parental stocks. From eggs receiving a dose of 1000*r* of X-rays no Red Eye pupated but 23% of Rock did pupate. However, 65% of the F_1 pupated. This resistance is probably due to heterosis rather than to a specific protective mechanism. From preliminary evidence, it appears that the rate of mitosis in the hybrids is higher and therefore repair of damage is more readily effected.

A STANDARD STRAIN OF *AEDES AEGYPTI*

The use of laboratory strains of insects of different origin and/or history of breeding as test insects for research on insecticides has often led to contradictory experimental data and conflicting hypotheses. In order to overcome this uncertainty, the World Health Organization has undertaken a programme aimed at providing standard strains of test insects, which should allow direct comparison of experi-

EVALUATION OF METHODS FOR DEVELOPING A STANDARD REFERENCE STRAIN OF *AEDES AEGYPTI*

Method	Advantage	Disadvantage
1. Conventional laboratory strain	Simplicity	(*a*) Marked variation among individuals (*b*) Change with time (*c*) Change in different laboratories
2. Inbred strain	Genetic uniformity	(*a*) Reduced viability (*b*) Some physiological responses variable (*c*) Cannot obtain large numbers
3. Selected inbred strain expanded	(*a*) Genetic uniformity (*b*) Hypothetically good viability	(*a*) Not currently available (*b*) Difficult to construct (*c*) Change with time, due to mutation
4. F_1 hybrid from two inbred strains	(*a*) Genetic uniformity (*b*) Excellent viability (*c*) Uniform physiological response (*d*) Minimal change with time	Cannot be used as breeding stock, due to breakdown of heterosis in F_2

mental data. For the housefly, a standard susceptible strain has been developed and is now available from Dr R. Milani, Zoological Institute, University of Pavia, Italy. It has been proposed that a WHO standard strain of *Aedes aegypti* be developed, perhaps by the University of Notre Dame.

The accompanying table summarizes four different ways whereby such a strain could be developed.

In considering these methods, No. 4 seems best for production of a standard strain of *Aedes aegypti*. Since eggs of this species can be stored for a year under proper conditions, large numbers of F_1 eggs could be accumulated and supplied to interested investigators on request. The investigator would not need to maintain a breeding colony because he could have a supply of eggs available for hatching whenever his experiments required them. Alternatively, samples of the two inbred lines could be furnished and the investigator could produce his own F_1 hybrids. No difficulty in shipping the standard strain is anticipated. Our laboratory has sent eggs of various strains all over the world in letter envelopes by air mail.

Heterosis is used in many areas of biology, its application in the production of hybrid corn being among the best known. The use of F_1 hybrids as experimental animals has been adopted in several areas of research. Modern workers in cancer research do their experiments on mice that are the F_1 from two inbred lines. Similar methods are used in plant and animal breeding, in immunology and in diverse areas of medical research.

GENETIC IMPROVEMENT

The great accomplishments of plant and animal breeders during the past 50 years show that organisms can be greatly modified through the deliberate action of man. Among entomologists, only workers with silk-worms and honey-bees have applied genetic methods to insect breeding. It is evident, however, that we do not have to accept mosquito species as they are today. One should be able to designate a form with certain desired characteristics, then construct that form through selective breeding. For mosquitos, with their short generation time, this should not be particularly difficult.

Very recently there has been much interest in autocidal control, the use of insects against themselves[1]. Autocidal programmes will generally require mass-rearing of insects under assembly-line conditions. Genetic methods must be applied in order to develop the most efficient and economical production methods and to control the quality of the end product.

In mosquito production, hybrid vigour should be a valuable tool. It has been shown that heterosis will

[1] Autocidal or genetic control is discussed more fully by the WHO Scientific Group on the Genetics of Vectors and Insecticide Resistance (1964) and by Craig (1963).

reduce development time and improve synchrony of pupation and emergence. This would speed up production and result in a more uniform product. The use to which the product will be put should regulate the genetic composition of mosquitos put into mass-production. If sterile males are desired, sterility can be built-in by breeding methods, thus avoiding the weakening effects of radiation or chemical sterilants. If certain behaviour traits such as high mating competitiveness are desired, these can be improved by selective breeding. Strains highly susceptible or highly resistant to pathogens, temperature extremes or insecticides can be constructed, in many cases using genetic material currently available. Genetic markers can be incorporated in strains to be released, thus facilitating field identification of released material. The possibilities of genetical manipulation of mosquito protoplasm for the good of man are extensive and much expansion in this area may be expected in future years.

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