Bull. Org. mond. Santé / *Bull. Wld Hlth Org.* 1967, **36**, 628-632

Genetic Control of *Aedes aegypti*

GEORGE B. CRAIG, JR [1]

It has been well established that pest insects can be genetically modified, produced in enormous numbers, released in the field and have a significant effect on field populations, even in subsequent generations. This is the rationale of genetic control. A WHO Scientific Group (1964) has defined genetic control as " ... the use of any condition or treatment that can reduce the reproductive potential of noxious forms by altering or replacing the hereditary material ". The subject has been reviewed by Downes (1959), Knipling (1960), LaChance & Knipling (1962) and Craig (1963a, 1963b).

In some ways, *Aedes aegypti* is a particularly promising subject for genetic control. First, our knowledge of the genetics of this species is developing rapidly, as described elsewhere in this number by Coker and by Craig & Hickey. Potentially useful genetic mechanisms have already been discovered, and more are sure to be. Secondly, the species has been mass-produced; it provides few obstacles to large-scale production or to field release of individuals bearing genetic characters that may be beneficial to man. Finally, certain characteristics of habitat and population dynamics are favourable for genetic control. Through most of its distribution, the species is closely associated with man in urban habitats. Thus, it is accessible. In addition, the generation time is short, and populations show periodic fluctuations in density. This should facilitate incorporation of released genetic material into populations at low ebb.

GENETIC MECHANISMS

Deleterious factors

The sterile-male technique is, of course, a method of genetic control; it involves production and dissemination of dominant lethal factors. Unfortunately, mosquitos treated with radiation or chemicals generally show reduced reproductive fitness. However, genetic breeding methods can be used to produce mosquitos that are sterile but reproductively competitive. Several genes conferring sex-limited sterility have already been isolated. The mutant *bronze* confers sterility on females of *A. aegypti* (Bhalla & Craig, 1964). Males are completely fertile; females are viable and fecund, but their eggs die about 12 hours after fertilization, due to a defective shell. Attempts to produce normal phenocopies by treatment of eggs with quinone-group chemicals are currently under way. At present, the stock is maintained by crossing ♀ *bz*/+ × ♂ *bz*/*bz* in every generation.

The mutant *intersex* confers sterility on males.[2] Larvae reared at 27°C give rise to normal males; however, rearing at 30°C feminizes males but has no effect on females. In *Aedes albopictus*, Bat-Miriam & Craig (1966) describe *proboscipedia*, a gene which converts the labella of the proboscis into tarsi. Males are fully fertile, but females are sterile because they cannot take blood.

In some mosquitos, agents for cytoplasmic incompatibility cause sterility in certain crosses. Laven (unpublished) has suggested that genetic control could be effected by mass release of males into an area with a population of incompatible crossing type. Such agents have been found in *Culex pipiens* and *Aedes (Stegomyia) scutellaris*, as well as in Drosophilidae and Chironomidae. As yet, no incompatibility has been found between geographic strains of *A. aegypti* (Hickey, 1961), but the potential value of this mechanism is such that the search should be continued.

In our own laboratory, crossing and inbreeding experiments have frequently revealed lethal genes, subvitals or sterility factors. These have generally been discarded. An organism must be rather well known genetically before genes for sterility or lethality can be manipulated. Such genes can be maintained by systems of balanced lethality or by phenocopy production, as are the nutritional mutants in *Neurospora*. We are now approaching this level of genetic sophistication in *A. aegypti*. As recommended by a WHO Scientific Group (1964), there should be a systematic effort to isolate and study lethal factors and other deleterious genes.

[1] Professor of Biology, University of Notre Dame, Notre Dame Ind., USA.

[2] See the paper by Craig & Hickey on page 559 of this issue.

Sex ratio

A genetic factor that distorts sex ratio in favour of males has been studied extensively by Hickey 1965a, 1965b). This male-producing (MP or Distorter) factor acts only in males and is passed to male offspring. The mechanism seems to be meiotic drive operating at or near the sex locus and causing selective production of male-determining sperm. Present strains give about 10% females. However, single crosses giving up to 130 males and no females from a single egg-batch are not uncommon, and MP strains with a lower proportion of females might be developed.

Experimental populations were established to follow the effect of MP over a series of generations. Females were exposed to various proportions of MP, and normal males and populations were allowed to breed continuously; sex ratio was determined at periodic intervals. In some populations a sex ratio of 10% female was maintained for more than 30 weeks. Combinations of 5 : 1 or 10 : 1 of MP to normal males were equally effective in maintaining continued distortion, whereas a 1 : 1 combination was somewhat less effective. It should be noted that a reduction from 50% female to 10% female is equivalent to 80% control of females. Moreover, this control was achieved by a self-perpetuating factor, since the population cages were undisturbed after the initial release.

Populations differ in their sensitivity to MP. Some strains are already homozygous for the factor; and release of MP males into these strains has no effect on sex ratio. In other strains, initial distortion of sex ratio is followed by a gradual return to normal, as insensitive chromosomes are selected. This development of "resistance" to MP required 14 generations in one experiment. In still other strains, no sign of insensitivity is evident at the end of 32 weeks of continuous rearing.

Small-scale field experiments can be performed with MP in the reasonably near future. As a first prerequisite, laboratory tests for MP sensitivity must be performed on the candidate field population. It is interesting to note that *A. aegypti mascarensis* from Mauritius is completely sensitive to MP.

The MP factor might be used to carry other genes through field populations. Von Borstel & Buzzati-Traverso (1962) have suggested that insect control could be achieved by synthesis and release of a meiotic drive chromosome containing a gene for female sterility. As the chromosome sweeps through a population, homozygous females would be useless for further propagation, but the males would still be produced in disproportionate numbers. Both a meiotic drive mechanism (MP) and a female-sterile mutant (*bronze*) are available in *A. aegypti:* these factors are about three crossover units apart on linkage group I.

Vectorial capacity

Genes conferring inability to transmit disease could be incorporated into field populations by mass release of laboratory-reared carriers, especially when such field populations are at a low ebb. A single gene makes *A. aegypti* refractory to infection with certain filariae (Macdonald, 1962) and another makes these mosquitos insusceptible to *Plasmodium gallinaceum* (Ward, 1963). It seems reasonable to assume that similar factors regulate vectorial capacity for virus diseases; the search for such factors would seem to deserve high priority in *A. aegypti* research. Until recently, technological difficulties in assaying virus content in single mosquitos made genetic studies impracticable. However, plant virologists have developed two methods; namely, the precipitin ring test of Withcomb & Black (1961), and the fluorescent antibody method of Nagaraj, Sinha & Black (1961), which should allow genetic work.

Genes that modify host-choice or biting habits might be useful. Macdonald, elsewhere in this number, reviews instances of *A. aegypti* populations that seldom attack man. It is at least conceivable that genes for zoophily could be substituted for those for anthropophily in field situations. Such a change has already occurred in certain *Anopheles* spp. as an accidental result of spraying programmes.

MASS PRODUCTION

Methods for large-scale production of *A. aegypti* have been developed in the USA by the Public Health Service (Morlan, Hayes & Schoof, 1963). Their techniques in the USA allowed 2 men to produce at least 650 000 adults every 8 days. This operation was on a pilot-plant basis. Assembly-line methods make expansion to factory-scale production entirely feasible. In this connexion, one must recall the record of the Florida screw-worm factory of the US Department of Agriculture, where 3 800 000 000 flies were produced in 22 months.

Genetic methods can be used to develop more efficient and economical methods and to control the quality of the end-product. For example, hybrid vigour gives both enhanced fitness and greater uni-

formity. In *A. aegypti*, heterosis will reduce development time, improve synchrony of pupation and emergence and enhance fecundity and longevity (Craig, 1964). Mass-production workers should use the F_1 hybrid of a cross between two inbred lines, as is done with hybrid corn, silkworms and many other agricultural organisms.

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 chemicals. If a release is be limited to males, the male-producing factor can be applied. If 90% of larvae hatching are male instead of the usual 50%, production capacity is automatically increased by 80%. Moreover, sex-linked mutants can be used to ensure early or late pupation of one sex. For example, crosses with the mutant *grey body* can be devised so that females do not begin to pupate until several days after males have emerged as adults (or the reciprocal). Strains highly resistant or highly susceptible to pathogens, temperature extremes or insecticides can be constructed, in some cases using genetic material currently available. Genetic markers can be incorporated into strains to be released, thus facilitating field identification of released material.

If certain behaviour traits, such as high mating competitiveness, are desired, these can be improved by selective breeding. Adhami & Craig (1965) have shown that single genes can affect mating competitiveness in *A. aegypti*. Moreover, it is the genetic constitution of the male that determines mating efficiency, the female having little choice of partners. *Drosophila* workers (for example, Manning, 1965; Connolly, 1965) have shown that traits such as mating speed and exploratory activity can be improved by selection. Schoenig (1965) has demonstrated genetic differences in lines of *A. aegypti* with regard to spontaneous activity. Research on behaviour genetics in *A. aegypti* deserves a high priority.

REPRODUCTIVE BIOLOGY

A major problem must be solved before genetic control of *A. aegypti* can be applied. Can laboratory-reared genetic material be introduced into field populations? Is the reproductive biology of this species consonant with the sterile-male method or other techniques of genetic control? The evidence to date is negative. Morlan, McCray & Kilpatrick (1962) released 4 770 000 radiation-sterilized males in Pensacola, Fla., but did not achieve any appreciable depression in field populations. Other workers have had similar results with *Anopheles quadrimaculatus* and *Culex fatigans*. Several hypotheses may be offered to explain these failures:

(1) *Mutagenic treatment may reduce mating competitiveness of males.* In the screw-worm case and in laboratory cages of *A. aegypti*, irradiated males were competitive. However, field behaviour of treated males has not been tested. Dame, Woodard & Ford (1964) showed that chemosterilant-treated males were not fully competitive.

(2) *Males from laboratory strains may not be able to compete successfully.* Cage-adapted strains probably show reduced mobility in the field. Dame et al. (1964) found that in *A. quadrimaculatus* males from a laboratory colony showed behavioural deficiencies in the field.

(3) *In the field, females may be inseminated initially by their own brothers from the same habitat.* If this is true, additional hypotheses may follow:

(*a*) *Field behaviour of mated females might make subsequent insemination by other males improbable.* Note, however, that in laboratory populations, multiple insemination is quite common, its frequency depending upon the strains used.

(*b*) *Sperms from subsequent matings may not be used in zygote production.* Again, this hypothesis does not hold true in the laboratory, There may even be a sperm-flushing effect, as in *Drosophila*, where results of an initial insemination may be obliterated by repeated subsequent matings that change the contents of the spermatheca.

If hypothesis (1) or (2) is correct, the condition may be modified. Sterility can be induced and high mating competitiveness can be established through agricultural breeding practices. If hypothesis (3) is correct, genetic control may not be feasible in this species, at least by way of released males.

An experiment should be performed to test these hypotheses. Genetically marked individuals should be released into field populations, and the populations should be sampled for several years to determine the fate of the released material in the field gene pool. This sort of experimental evolution has been done extensively with *Lepidoptera*, *Drosophila*, snails and mice (Ford, 1964). These studies have shown that genetic factors can not only be incorporated into field populations, but that, in certain cases, such factors will sweep through a population and become predominant, even though this pre-

dominance is actually disadvantageous to the species (Anderson, Dunn & Beasley, 1964).

The proposed experiment should be performed in an island situation with an abundant population of *A. aegypti*. About 20 of the 80 or so mutants currently available would be useful as markers. Stocks should be constructed with one or two mutants and maximum genetic material from the test-site population. Releases would be made from different sites, using a different marker for each site.

Three types of release should be made. First, males should be reared in the laboratory and released near breeding-sites (as in the sterile-male method). Secondly, eggs and larvae should be inserted into field habitats, so that adult emergence is synchronized with field populations. Thirdly, field larvae should be collected and reared, emerged females should be inseminated by marked laboratory males, and these females with marked sperm in the spermatheca should then be released in the field. For several years following release, field populations would be collected and tested for presence of the markers.

If the released material were retained in field populations, one could then proceed to investigate the prospects for genetic control. The use of sterility factors (spontaneous or induced), meiotic drive, lethal genes, sex-ratio distorters or genes affecting vectorial ability would then be feasible. If the released material disappeared very quickly, or if field females remained uninseminated by marked males, genetic control of *A. aegypti* would be contra-indicated. However, the results would still be of interest. They would then show that, contrary to the belief of some eradicationists, laboratory colonies do not endanger eradication programmes, since laboratory escapees, at least of the strains tested, could not become established in the field.

CONCLUSION

Genetic control of *A. aegypti* may be feasible. The number of research workers in this field is negligible, yet promising genetic mechanisms have been discovered already. However, additional research is needed on sterility and lethal factors, on genetics of behaviour and on sexual behaviour and mating competitiveness. Field experimentation is particularly important. In addition, research is needed on ecology and population dynamics in order to discover how, where, when and in what numbers mosquitos should be released for genetic control. Will genetic control work? It is not known. Yet the need for non-chemical approaches to control is such that any potential concept should be prosecuted vigorously.

REFERENCES

Adhami, U. M. & Craig, G. B (1965) *Mating competitiveness in* Aedes aegypti. In: Freeman, P., ed., *XIIth International Congress of Entomology, London, 8-16 July, 1964 (Proceedings)*, London, Royal Entomological Society of London, p. 260

Anderson, P. K., Dunn, L. C. & Beasley, A. B. (1964) *Amer. Nat.*, **98**, 57

Bat-Miriam, M. & Craig, G. B., Jr (1966) *Mosquito News*, **26**, 13

Bhalla, S. C. & Craig, G. B., Jr (1964) *Bull. Ent. Soc. Amer.*, **10**, 173

Connolly, K. (1965) *Selection for exploratory activity and its effects on other aspects of behaviour in* Drosophila (Diptera). In: Freeman, P., ed., *XIIth International Congress of Entomology, London, 8-16 July, 1964 (Proceedings)*, London, Royal Entomological Society of London, p. 258

Craig, G. B., Jr (1963a) *Bull. Wld Hlth Org.*, **29**, Suppl., p. 89

Craig, G. B., Jr (1963b) *Proc. N.C. Branch Ent. Soc. Amer.*, **18**, 13

Craig, G. B., Jr (1964) *Bull. Wld Hlth Org.*, **31**, 469

Dame, D. A., Woodard, D. B. & Ford, H. R. (1964) *Mosquito News*, **24**, 1

Dame, D. A., Woodard, D. B., Ford, H. R. & Weidhaas, D. E. (1964) *Mosquito News*, **24**, 6

Downes, J. A. (1959) *Canad. Ent.*, **91**, 661

Ford, E. B. (1964) *Ecological genetics*, New York, J. Wiley

Hickey, W. A. (1961) *Bull. ent. Soc. Amer.*, **7**, 175

Hickey, W. A. (1965a) *Distortion of sex ratios in* Aedes aegypti *(Diptera: Culicidae)*. Notre Dame, Ind. (Thesis, Notre Dame University)

Hickey, W. A. (1965b) *Genetic distortion of sex ratios in* Aedes aegypti. In: Freeman, P., ed., *XIIth International Congress of Entomology, London, 8-16 July, 1964 (Proceedings)*, London, Royal Entomological Society of London, p. 266

Knipling, E. F. (1960) *J. econ. Ent.*, **53**, 415

LaChance, L. E. & Knipling, E. F. (1962) *Ann. ent. Soc. Amer.*, **55**, 515

Macdonald, W. W. (1962) *Ann. trop. Med. Parasit.*, **56**, 373

Manning, A. (1965) *Some behavioural selection experiments with* Drosophila *(Diptera)*. In: Freeman, P., ed., *XIIth International Congress of Entomology, London, 8-16 July, 1964 (Proceedings)*, London, Royal Entomological Society of London, p. 257

Morlan, H. B., Hayes, R. O. & Schoof, H. F. (1963) *Publ. Hlth Rep. (Wash.)*, **78**, 711

Morlan, H. B., McCray, E. M. & Kilpatrick, J. W. (1962) *Mosquito News*, **22**, 295

Nagaraj, A. N., Sinha, R. C. & Black, L. M. (1961) *Virology*, **15**, 205

Schoenig, E. (1965) *Strain variation in behavior in* Aedes aegypti. Notre Dame, Ind. (Thesis, University of Notre Dame)

Von Borstel, R. C. & Buzzati-Traverso, A. A. (1962) *On the role of lethal mutants in the control of populations.* In: *Radioisotopes and Radiation in Entomology : Proceedings of a Symposium, Bombay, 5-9 December 1960*, Vienna, International Atomic Energy Agency, p. 273

Ward, R. A. (1963) *Exp. Parasit.*, **13**, 328

Whitcomb, R. F. & Black, L. M. (1961) *Virology*, **15**, 136

WHO Scientific Group on the Genetics of Vectors and Insecticide Resistance (1964) *Wld Hlth Org. techn. Rep. Ser.*, **268**, 20