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The Establishment of Reference and Marker Strains and Their Shipment

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The World Health Organization convened a Scientific Group on Standardized Strains of Insects of Public Health Importance in July 1965. This Scientific Group considered all facets of the problem, including the establishment of reference and marker strains of *Aedes aegypti* and their shipment. This paper embodies the views of the Scientific Group.

DEFINITIONS

Standardized strains of insects are prepared, using genetical methods with a particular purpose in mind as to their ultimate use. Standardized strains may be divided into a number of types, as discussed below.

Inbred strain

An inbred strain is one that is developed by close inbreeding so as to make it, as far as possible, isogenic. The Scientific Group recommended that the term " inbred strain " be reserved for strains that have been developed and maintained at a level of at least 95% homozygosity.

F_1 hybrid

An F_1 hybrid is one produced by crossing two inbred lines. It is useful for its heterosis, which is expressed in its excellent viability and uniform physiological response. It is only intended for use as such, since all the advantages gained as hybrids are lost through recombinations in subsequent generations.

Reference strains

Reference strains are those with reduced genetic variability. It is essential that these be homozygous for some specific characteristic. They have been subdivided, for the present, into the following three categories.[2]

(1) *Insecticide reference strain :* one that has been selected for homozygosity for susceptibility or resistance to an insecticide.

(2) *Species reference strain :* one that has been selected for homozygosity for genes that control isolating mechanisms among closely related species.

(3) *Incompatibility reference strain :* one that has been selected for a certain cytoplasmic crossing type that controls fertility.

Marker strains

Marker strains are strains which carry specific mutant markers. One or more linkage groups may be marked with one or more genes. However, a mutant should only be used as a marker if it is easily seen, is fully viable and has full penetrance and expressivity.

DEVELOPMENT OF STANDARDIZED STRAINS

The strains of *A. aegypti* described below are largely those in existence at the University of Notre Dame, Notre Dame, Indiana, USA, where they have been developed by Professor G. B. Craig, Jr and his associates (see table). Many other strains of *A. aegypti* exist in other laboratories which have been selected for specific purposes and could admirably serve as standardized strains of some type. The Scientific Group has recommended that as many of these strains as possible be investigated by WHO as to their suitability for use as standardized strains. It would be greatly appreciated if the participants would communicate to WHO any information on this subject.

Inbred strains

Several inbred strains of *A. aegypti* are maintained at Notre Dame by single-pair, brother-sister mating in each generation. The generation number of each strain is given following the strain

[1] Vector Biology and Control, World Health Organization, Geneva, Switzerland.

[2] The Scientific Group indicated that the above list of reference strains was by no means complete, and that in future it would be necessary to set up reference strains for such characteristics as vector ability, chromosome patterns, biochemical characteristics and behaviour types.

STRAINS OF *AEDES AEGYPTI* AVAILABLE FROM THE MOSQUITO GENETICS PROJECT, UNIVERSITY OF NOTRE DAME[a]

I	*A. aegypti* - Geographic	
	A. Type form - 9	(GANDA, ROCK, KUALA, MICK, MANDALAY, NIH, NEWX, KENYA, NESS)
	B. Variety *queenslandensis* - 1	(AO)
	C. *A. a. formosus* - 8	(BRAZZA, BRAZZA-Y, ILOBI, SSISA, DAHOMEY, GKPE, TANA, YAOUNDE)
II	*A. aegypti* - inbred - 7	(RED-F_{16}, KH-F_{17}, GKPE-F_{13}, BLPCO-F_{14}, NESS ABC-F_{60})
III	*A. aegypti* - Mutants	
	A. Linkage group I - 13	(including *re, ru, w, bz, N, br* and sex locus)
	B. Linkage group II - 15	(including *G, y, s, Si, bpd, ds, wa, sp, ix, wp, h*)
	C. Linkage group III - 10	(including *fz, blp, co, blt, min, wi, th, anb*)
	D. Multiple marker - 6	(markers on all three chromosomes)
	E. Linkage unknown - 9	(including *B, bu, k, bn, kl, Fl, buff, grey*)
IV	Other *Aedes*	
	A. *Finlaya* *triseriatus* *togoi* *sierrensis* - 2 *atropalpus*	(including *yellow*)
	B. *Stegomyia - albopictus* - 9	(including mutants, *y, blp, prs, wp, bu* and autogeny)
	C. *Stegomyia - mascarensis* - 3	
	D. Other *Stegomyia* *vittatus* *pseudoscutellaris*	

[a] The Project is under the direction of Professor G. B. Craig, Jr. Numbers indicate numbers of strains. Some strains are available with as many as five mutants on a linkage group.

designation. This generation number is subject to change. The symbols given are those used by Craig.

RED, F_{15}	Homozygous for *re* (I), *s* and *g* (II), *blt* (III).
KH, F_{15}	Homozygous for *sp* (II).
GKPE, F_{12}	Subspecies *formosus*.
BLPCO, F_{10}	Homozygous for *blp* and *co*, both on linkage group III.
NESS-A, F_{60}	Homozygous for *wp* (II). Larval colour yellow.
NESS-B, F_{60}	Homozygous wild type. Larval colour dark.
NESS-C, F_{60}	Homozygous wild type. Larval colour dark.

Insecticide reference strains

Several strains have been selected for DDT and dieldrin susceptibility and/or resistance.[1]

Marker strains

About 80 mutants of *A. aegypti* have been isolated; the majority were found at and are available from the Mosquito Genetics Project, University of Notre Dame. About 25 genes are useful as chromosome markers. Most of these mutants are maintained in strains homozygous for three or four mutants on a single linkage group.

Complete lists of stocks (geographic, taxonomic,

[1] A listing of these strains may be found in the *WHO Information Circular on Insecticide Resistance*, Supplement B, January 1963.

inbred strains, mutants, markers) plus a list of mutants, are available as mimeographed documents from the Mosquito Genetics Project, University of Notre Dame, Notre Dame, Indiana, USA.

A. aegypti is especially important in view of the transmission of arboviruses and the recent outbreaks of haemorrhagic fever in some parts of the world. Considerable effort has been put into developing a standardized strain of this species, and it is fortunate that inbred and marker strains have been synthesized. Insecticide reference strains have also been at least partially developed. All components exist for the production of F_1 hybrid strains that would be of great value for experimental purposes. Experiments have already demonstrated useful levels of heterosis in *A. aegypti*.

Since eggs of *A. aegypti* 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 investigators would not need to maintain a breeding colony because they could have a supply of eggs available for hatching whenever their experiments required them.

The Scientific Group strongly recommended investigating the possibility of encouraging the production of the F_1 hybrids and, if this production is possible, making these hybrids available to research workers, as has been done with other standardized strains.

SHIPMENT

The transport of *A. aegypti* eggs does not present a problem. Eggs can be dried on a filter-paper and sent through normal channels. The filter-papers with desiccated eggs are placed in a plastic or aluminium-foil bag.

Adult mosquitos can also be transported in protective boxes, with the provision of proper humidity and food. A polystyrene box has been found to be very satisfactory. The advantage of a polystyrene surface is that it absorbs excessive moisture, and mosquitos do not stick to the surface of the box. Further development of this type of box and its manufacture is engaging the attention of WHO.

However, the ideal method of shipping *A. aegypti* strains is by eggs.