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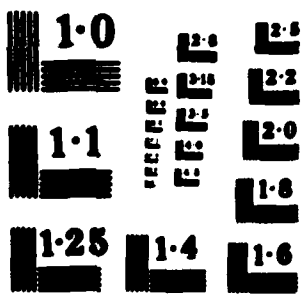
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Genetic Characterization of Insect Vectors of Disease

Annual Report

Jeffrey R. Powell

September 1, 1981

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

We have carried out extensive population genetic studies on the yellow fever mosquito, Aedes aegypti, concentrating on populations from the southern U.S. The goal of the research is to define genetic-geographic groupings of this serious insect vector.

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Because of the timing of the review panal's meeting, I hope the members will bear in mind that this "annual" report is really a semi-annual report. The contract under which the work was done has been in effect only six months.

WORK ACCOMPLISHED

The proposed projects involved population genetic analysis of Aedes aegypti. The main technique employed is gel electrophoresis of soluble proteins followed by staining for specific enzyme activity. Using this method we have identified 22 gene loci in this mosquito. Twelve of the 22 loci are variable (polymorphic), ten of which are very variable and useful for our studies. The objectives of the research is to study allelic variation at these variable loci in order to answer the following questions about A. aegypti: (1) How genetically variable are populations? (2) How much genetic variation exists between populations? (3) Can we define areas within the distribution of A. aegypti which have populations genetically similar and differentiated from other areas? (4) Do the world-wide patterns of genetic variation in this vector correspond in any way to patterns of diseases transmitted by A. aegypti? Rather than go into great detail here on the results obtained before this contract was in effect, the reader can consult the original proposal and the following references: Tabachnick and Powell, 1978, 1979; Tabachnick et al., 1979, Powell et al., 1980. Here we shall be concerned only with new data collected under this contract and not yet published.

Based on the results previously obtained we concluded that A. aegypti populations in the New World exhibited a fascinating and complex pattern of genetic variation. Originally we noted that the New World had three distinct, genetically

differentiated areas: S. America, the Caribbean, and the Southeast United States (as far west as New Orleans). Most surprising was the rather large differentiation between the Caribbean and Southeast U.S. We speculated at that time that this may explain why dengue had not gotten into the Southeast U.S. Much like Dudley (1934) had speculated many years ago with respect to Asia and yellow fever, it is possible that the genotypes of A. aegypti inhabiting the Southeast are less efficient transmitters of dengue viruses. To be sure there is no direct evidence of this, but it seemed worthy of further consideration. One question which remained was what genetic type of A. aegypti was inhabiting Mexico and Texas? At that time we had but one Texas collection (Laredo) and one Mexcan (Piedras Negras); they both were clearly very similar to the Carribean types. If our hypothesis is correct, than, knowing that Caribbean A. aegypti are good vectors of dengue, we further speculated that Mexico and at least Laredo were in danger of having dengue. This speculation was borne out when dengue did appear in Mexico and just last fall the first cases of dengue in the U.S. for many years were detected in Laredo (and Brownsville).

While all of the limited data at that time was consistent with the hypothesis, we emphasized it was very limited indeed. The whole story was based on collections from only 11 localities: 5 Caribbean from only two islands (Jamaica and Puerto Rico), 2 S. American (both from Venezuela), 3 Southeast U.S. collections (Florida and New Orleans), 1 Texas, and 1 Mexican. Therefore the major goal of the proposed research was to clarify these patterns by adding more collections from the New World. One question of paramount importance was what is the pattern between Laredo and New Orleans? Also, what is happening on other Caribbean Islands? In addition to concentrating on the New World we were to continue analysing samples from thoughout the world as they became available through workers in the field.

Table 1 lists the samples from the New World and from elsewhere which have been

analysed since the start of this contract. A total of 29 independent samples from 17 different localities have been studied. (As pointed out in the original proposed, if at all possible we work with freshly collected material and aim for a sample size of 100-200 genes locus from the natural population. The majority of our samples meet these criteria but on occasion we must be content with whatever we are able to procure; these exceptions will be pointed out later.) Table 2 lists 5 samples from these localities which are presently in our lab undergoing analysis. Thus within a few weeks of this writing, we will have more than doubled the number of localities in the New World which have been studied for genetic variation in A. aegypti populations. Also listed in Table 2 are localities where we have contacted mosquito workers who have agreed to send as collections, hopefully quite soon.

TENTATIVE CONCLUSIONS

Because this work is ongoing, we will not present any firm conclusions nor go into any detailed analyses. We have done preliminary analyses which include both genetic distance matrices and a stepwise linear discriminant analysis of the data obtained to date; the following disussion is based on these. (The actual analyses are not presented here as they are rather cumbersome at this point; if the committee would like to examine the several pages of computer print-out, we will supply them.)

With the additional localities now sampled, our main conclusion is that the picture in the New World is not as simple as we originally thought. We now realize that there are at least five distinguishable genetic-geographic regions in the New World. These are (1) Southeast U.S., (2) Texas-Mexico-Central America, (3) Northern Caribbean, (4) Trinidad-Northeast S. America Coast, (5) Other S. American localities. While these conclusions are only tentative at this time, four aspects of these results warrant further discussion.

First, while originally our two Texas-Mexico populations were indistinguishable from Caribbean populations (Jamaica and Puerto Rico), with the additional samples these groups become recognizably different, although still very similar genetically. They are still much more similar to each other than either is to the Southeast U.S. The single Central American collection, Guatemala, falls into the Texas-Mexico group.

Second, with two exceptions all of the new Texas samples fall into the Texas-Mexico group. One exception is the Corpus Christi sample; this sample was also exceptional in that it was a laboratory colony (obtained from Robert Novak, CDC). Thus although interesting we do not put much faith in it. The second exception was Weslaco which is close to Brownsville. At present we really don't understand this, as populations to the south, west, north, and northeast all do fit into the Texas-Mexico group. We have not been able to ascertain how our Weslaco sample was obtained since information from the collectors who sent it to us has not been forthcoming. However, we are currently analysing a fresh collection from Corpus Christi and anticipate additional collections from Brownsville so we will check these anomalies. If this pattern is indeed proven, we see that the transition from Texas-Mexico type to Southeast U.S. type probably occurs between Galveston and New Orleans, a very short distance.

Third, the Caribbean is heterogeneous. Trinidad is clearly distinct from Jamaica and Puerto Rico, and is very close (genetically) to Surinam. This is not too suprising since Trinidad is also very close geographically to the northeast coast of S. America. Thus further collecting in the Caribbean is warranted to learn how heterogeneous it is and where the transitions occur.

Fourth, southeast U.S. populations are more closely related to West and East African *A. gambiae* complex than to any other group. This was missed in our earlier analysis due to the lack of enough samples with which to draw comparisons.

While based on morphology, the majority of Southeast U.S. A. aegypti would not be considered subspecies formosus, the isozyme data however do reveal similarities. This may be significant for the following reason. In yellow fever infectivity tests, the infectivity was low in both African formosus subspecies and New Orleans A. aegypti. (Gubler, personal communication; and our own collaboration with YARU). This implies that the genes controlling the morphological differences between subspecies, may not be marking the genome as well as our isozyme markers with respect to genetically determined variation in yellow fever infectivity. Admittedly we have a long way to go in proving hat the isozyme markers will be useful in studying the genetics of vectorial capacity. However, as we do our population surveys, we keep finding encouraging patterns which we feel deserve serious consideration.

THE NEXT SIX MONTHS

As noted above, we still have six months remaining on the first year of this contract. During that time we will accomplish three things:

1. More population samples will be sent to us and we will analyze them. A few of the places where we expect samples are listed in Table 2. Also we have made contacts and inquiries elsewhere and expect a few others besides these.

2. Make a collecting trip to the Southern U.S. Orignally we had proposed to make a trip to Texas to study the transition from the Texas-Mexico type (then only known from the vicinity of the Rio Grande) to the Southeast U.S. type found in New Orleans. We postponed this trip to see if we could get more Texas samples from field workers. This would enable us to concentrate our collecting when we went to Texas, as it was obvious we couldn't cover the whole state. We will probably wait until the beginning of October in order to obtain a few more samples. At present it appears that the rather small area between Galveston and New Orleans is the most important area. However we also must clarify the Weslaco/Brownsville and Corpus

Christi problem alluded to above. If more samples are not sent from these localities we will have to include them in our trip.

3. Make a collecting trip to the Caribbean. Again, depending on what samples we receive from the Caribbean during the next few months, we will decide on exactly where to go. It is clear there are relatively large differences between northern islands (represented by Jamaica and Puerto Rico) and at least one southern island, Trinidad. Where does the transition occur? Also, when the data are in on this year's dengue outbreaks in the Carribbean, we may be able to choose islands of interest in that respect -- after the dengue season is over! The question would be: Is there any pattern of genetic variation in A. aegypti which correlates with dengue outbreaks?

U.S. ARMY INTERACTION

As stated in the original proposal we have maintained contact with personnel at Fort Detrick. We have advised them of the collections we have received, what data we have collected on them, and offered to send them whatever material they may find useful. We intend to continue this practice.

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Table 1. Populations of *A. aegypti* from the New World and elsewhere, analysed for genetic variation during six months of this contract.

Locality	Abbreviation	No. of Independent Samples
NEW WORLD:		
Galveston, Texas	GALTEX	3
Austin, Texas	AUSTEX	1
San Antonio, Texas	SANTEX	1
Corpus Christi, Texas	CCTEX	1
Eagle Pass, Texas	EAGTEX	1
Weslaco, Texas	WESTEX	1
Montemorelos, Mexico	MONMEX	1
Victoria, Mexico	VICMEX	2
Guatemala	GUAT	1
Paramaribo, Surinam	PARA	2
Trinidad	TRIN	1
ELSEWHERE:		
Majengo, Kenya	MAJ	1
KWA Dzivo, Kenya	KDZ	1
Bwerenga, Uganda	BWE	1
Accra, Ghana	ACCRA	1
Kedougou, Senegal	KED	4
Dakar, Senegal	DAK	6

Table 2: Populations of A. aegypti presently being analysed and those we anticipate receiving within a short time.

Locality	Abbreviation	No. of Independent Samples
UNDER ANALYSIS:		
Beaumont, Texas	BEAUTEX	1
Trinidad	TRIN 2,3,4	3
Corpus Christi, Texas	CCTEX 2	1
ANTICIPATED:		
Brownsville, Texas		
McAllen, Texas		
Houston, Texas		
Port Arthur, Texas		
Austin, Texas		
Nederland County, Texas		
Quintana Roo, Mexico		
Costa Rica		
Dominica, West Indies		

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