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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 10/19/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose VO

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 3. F-50
First Annual Report dtd May 85
+ Dif. Mo. PR of year 3 rec'd 1/30/87

Attachments

2

PD-AAW-690
3. F-50

SIX-MONTH PROGRSS REPORT OF YEAR THREE

ON

BIOLOGICAL CONTROL OF SCHISTOSOMIASIS TRANSMITTING SNAILS IN SOUTHEAST ASIA

SUBMITTED TO

OFFICE OF THE SCIENCE ADVISOR
PROGRAM IN SCIENCE AND TECHNOLOGY COOPERATION

BY

DR. SUCHART UPATHAM
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RAMA 6 ROAD
BANGKOK 10400
THAILAND

GRANT NUMBER 936-5542
DECEMBER 1986

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I. BACKGROUND

The award of the project entitled "Biological Control of Schistosomiasis - transmitting Snails in Southeast Asia" was presented by Mr. John Gunther Dean, Ambassador of the United States of America to Thailand, to Professor Dr. Natth Bhamarapravati, Rector of Mahidol University, at the Rockefeller Foundation Conference Room, Faculty of Science, Mahidol University, on 5 March 1984. The first payment of the grant was made available to the Faculty of Science in early April 1984. Four reports has been submitted, i.e. the six - month report of year one, the annual report of year one, the six - month report of year two and the annual report of year two. This is the six - month report of year three covering the period from May to November 1986.

II PROJECT OBJECTIVE

The overall objective of this research project is as follows:

1. to study the efficacy of biological control of snails transmitting schistosomiasis in Southeast Asia using biological agents, and

2. to study genetic variability of snails transmitting schistosomiasis in Southeast Asia in relation to degrees of parasite compatibility and susceptibility.

III EXPERIMENTAL GOALS

1. Laboratory experiments. These results will include the biological aspects of insects and snail agents and their efficacy against the target snail species. The life histories of insects and snail agents will be studied and various doses of biological agents and target species will be tested. This phase of study will take approximately 16 months.

2. Simulated field experiments. Biological agents and target species will be tested in simulated field habitats. This phase of study will take approximately 8 months.

3. Genetic and susceptibility studies. Genetic variability of snails transmitting schistosomiasis in relation to degrees of parasite compatibility and susceptibility will be studied under laboratory conditions. This phase of study will take approximately 3 years.

4. Small scale field experiments. Pending the results of "goals" one and two as delineated above, a small scale field trial will be initiated. These experiments will include only local target species. This last stage of study will take approximately one year.

IV RESEARCH FINDINGS

1. Biological control of snails

1.1 Mollusks

According to the results obtained from the experimental work on biological control of mollusks in the

laboratory and under simulated field conditions, we decided to choose *Pila ampullacea* as the control agent for a small scale field trial experiment. *P. ampullacea* appeared to be a very suitable control agent due to their large size and their voracious habit. According to the data obtained from previous experiments, there was a reduction in growth and reproduction of the snail target species, such as *Radix rubiginosa, Indoplanorbis exustus,* and *Biomphalaria glabrata.* These effects were based on competition for food, physical interference between snails as predator on egg masses of the target species.

The study has included only local target species of snails, such as *Indoplanorbis exustus, Radix rubiginosa* and *Bithynia siamensis goniomphalos.*

The small scale field trial experiment was conducted at a local field station outside Bangkok where there were natural canals. The canal selected was 15 meters long and 1 meter wide. It was divided into 7 partitions, each of the size of 2x1 squaremeters, by using the nylon net hang over the wooden frames. There were natural aquatic plants in the canal, such as water hyacinths, *Elodea* and water lettuce.

The ratios between biological agent and snail target species were as follows:

1st partition	100 *P. ampullacea*	
	300 *I. exustus*	

2nd partition	100	*P. ampullacea*
	300	*R. rubiginosa*
3rd partition	100	*P. ampullacea*
	300	*B.s. goniomphalos*
4th partition	100	*P. ampullacea*
5th partition	300	*I. exustus*
6th partition	300	*R. rubiginosa*
7th partition	300	*B.s.goniomphalos*

The general procedures, observation on mortality rates of both control and target species and the counting of the snail offspring were done biweekly. The original snails were marked with finger nail polish so that the offspring could be distinguished from them.

The small acale field trial experiment is in progress, and the results will be reported later on.

1.2 Insects

Sciomyzid fly larvae were used as biological control agent against snails. According to laboratory and simulated field data , the best results of controlling pulmonate snails by sciomyzid fly larvae were obtained at the ratio of 1 : 3 (snails : sciomyzid fly larvae). Therefore, the ratios of sciomyzid fly larvae to pulmonate snails selected for this small scale field trial experiment were

4:0, 3:1, 3:2 and 0:4.

The experiment was conducted at a local field station outside Bangkok where there were natural canals, as mentioned in Section IV (1.1). Each of the cages used measured 1m long, 1m wide and 2m high. Nylon net was used to cover the wooden frame cages. Part of the cage (30 cm) was placed under the water.

Observation on mortality rates of both control and target species was done biweekly.

The experiment is in progress, and the results will be reported later on.

2. Genetic studies

2.1 Genetic variation and differentiation of three *Schistosoma* species from the Philippines, Laos and Peninsular Malaysia (Figure 1).

Electrophoretically detected allozyme variation is described in strains of *S. japonicum* (4 Philippine strains), *S. mekongi* (Laos), and an undescribed anthropophilic *S. japonicum* -like schistosome from Peninsular Malaysia. Results, together with those reported previously for 8 other strains (*S. japonicum* - China, Formosa, Japan, Philippines; *S. mekongi*- 2 substrains; Malaysian schistosome - 2 strains) permit a composite genetic characterization of 15 strains of

Asian schistosomes at 9 - 18 presumptive loci. The proportion of polymorphic loci (P) and the mean heterozygosity per locus ($\bar{H}$) were zero in all strains. Although this was expected for strains that had been in laboratory culture for up to 50 years, we expected to detect variation in strains based on 10 - 50 recently field-collected infected snails. we expected *S. japonicum* to be as variable as *S. mansoni* ($\bar{P}$ = 0.13 (0-0.33), $\bar{H}$ = 0.04, 18 loci, 22 strains) as it believed to reporduce sexually, has an evolutionary history of several million years, inhabits a wide geographic range, coevolved with a genetically variable intermediate host snail, and has a diversity of mammalian hosts.

No differences were detected between the 5 *S. japonicum* strains from Leyte and Luzon (Philippines), between the 3 *S. mekongi* strains, or between the 3 Malaysian schistosome strains; these groups and the remaining *S. japonicum* strains representing Mindoro (Philippines), China, Formosa and Japan each have distinctive multilocus electromorphic patterns. NEI's genetic distances (D) were calculated to estimate interstrain and interspecific divergence. Interstrain genetic distances in *S. japonicum* averaged > 0.3; much higher than those reported previously for *S. mansoni* ($\bar{D}$= 0.06, D (max) = 0.24). *S. japonicum* (Mindoro) was moderately differentiated from the Leyte-Luzon strains ($\bar{D}$ = 0.29, 12 loci). Estimates of the *S. japonicum* China - Philippine distance (D > 0.4, 11 loci) are high for conspecific

populations and further studies of the still poorly characterized Chinese parasite may reveal that these are, in fact, separate species. *S. japonicum* is shown to be only distantly related to *S. mekongi* and the Malaysian schistosome ($D > 1$); the latter is closely related to, but genetically quite distinct from, *S. mekongi* ($D = 0.61 \pm 0.275$, 11 loci) and warrants recognition as a new species.

This report has the following medical and public health consequences. First, the possible medical correlates of the increasingly complex taxonomic situation merit more attention than they have received. It is reasonable to expect the different species and species groups of schistosomes to differ in their epidemiology, pathology and response to various treatments. Second, our continued failure to find intrastrain variation in the Asian schistosomes has important ramifications. Although isogenic strains are ideal for some investigations (immunological experiments and sources for monoclonal antibodies), single isogenic strains may be inappropriate models for geographically widespread species. Development of broadly applicable disease diagnosis, treatment and control techniques involving vaccines and drugs may be more difficult in view of this pattern of marked geographic variation (Woodruff, 1985). There is, after all, apparently much more regional differentiation within *S. japonicum* ,as presently defined, than within *Homo sapiens*.

2.2 Genetic differentiation of the Chinese *Schistosoma japonicum* from the *S. japonicum* species complex.

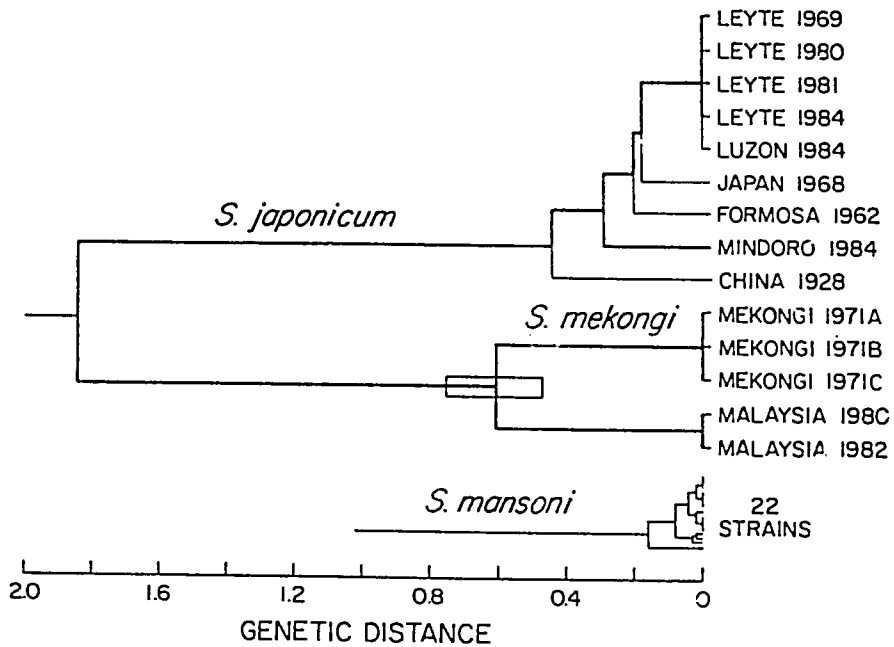

Figure 1. Dendrogram showing the relationships of some Asian schistosome strains, generated according to UPGMA cluster analysis and based on 11-15 loci (thick lines) or 7-10 loci (thin lines). For comparative purposes, a schematic outline of the tree for *S. mansoni* (18 loci) is shown below.

The first genetic evidence suggesting that the Asian anthropophilic human blood flukes presently refered to as *Schistosoma japonicum* may actually comprise a group of sibling species was provided by Fletcher *et al.* (1980). In a study providing genetic evidence that *S. mekongi* is a separate species from *S. japonicum*, they compared laboratory strains of *S. japonicum* originally from China, Taiwan, Japan, and the Philippines and found the Chinese strain to be the most distinct. Multilocus indices of genetic differentiation for the Chinese-Philippines comparison were suprisingly high for a conspecific comparison (e.g. Nei's D = 0.45, Fletcher *et al.*, 1980); a comparable study of variation between 22 strains of *S. mansoni* yielded $\bar{D}$ = 0.06, Dmax = 0.024 (Fletcher *et al.*, 1981). This preliminary study was inadequate, however, as only 10 loci were studied and as the Chinese worms, laboratory descendents of Vogel's 1928 Kofu Province collection, were possible unrepresentative of naturally occurring schistosomes. A subsequent study of 12-18 loci (Woodruff *et al.*, 1986a) confirmed the species status of both *S. mekongi* and of an undescribed anthropophilic Malaysian schistosome. In the case of *S. japonicum*, they found no difference between strains from Leyte and Luzon in the Philippines, and moderate differentiation (D = 0.029) of a strain from Mindoro, Philippines. The purpose of the present note is to report comparative data for a recently established Chinese strain. We find that *S. japonicum* strains from China and the Philippines are even more

differentiated from one another that suspected; the taxonomic and medical significance of this discovery are discussed.

Approximately 40 infected *Oncomelania hupensis hupensis* (natural intermediate host snail) were collected in February, 1984 from Guichi, Anhui Province, Peoples Republic of China (on the Yangtze River approximately 400 Km west of Shanghai) and 50 infected *O.h. quadrasi* were collected in 1984 from Victoria, Mindoro, Philippines. Cercariae of each strain were cycled through Swiss Webster mice once. All schistosomes were cultured at the Center for Applied Malacology and Entomology, Department of Biology, Faculty of Science, Mahidol University, Bangkok. After perfusion of the adult parasites from the mice and tissue preparation, starch gel electrophoresis was performed is San Diego according to techniques described in Fletcher *et al.* (1980) and Woodruff *et al.* (1986a). We resolved GAP (E.C. 1.2.1.12) and MDH-1 (E.C. 1.1.1.37) using continuous tris-citrate (pH 7.0) buffer; G6PD (E.C. 1.1.1.49), HK (E.C. 2.7.1.1) and ME (E.C. 1.1.1.40) using citrate with aminopropylmorpholine (pH 6.0) buffer; MDH-2 (E.C. 1.1.1.37), ACP (E.C. 3.1.3.2), PGM (E.C. 2.7.5.1) and PGD (E.C.1.1.1.49) using tris-malate (pH 7.4) buffer; PEP-lgg (leucylglyclyglycine substrate) (E.C. 3.4.1.3), MPI (E.C. 5.2.1.8), and GPI (E.C. 5.3.1.9) using citrate with aminopropylmorpholine (pH 7.0) buffer; ALD (E.C. 4.1.2.13), GDH (E.C. 1.4.1.3), LDH (E.C. 1.1.1.27) were resolved only by Fletcher *et al.* (1980). We here report results for 58 individual male worms from the mainland of

China and 66 males from the Philippines. For technical reasons associated with the size of the schistosomes, we scored an average of 10 individuals per locus. To ensure reliability of the results, we ran all procedures twice.

The electromorph patterns obtained are reviewed in Woodruff *et al.* (1986a). The proportion of polymorphic loci (*P*) and the mean individual heterozygosity per locus (*H*) are zero for both strains. These results are in concordance with Fletcher *et al.* (1980) and Woodruff *et al.* (1986a). Overall genetic difference between the two strains can be estimated using an unbiased multilocus genetic distance: Nei's genetic distance (*D*) (1978). Between *S. japonicum* from the mainland of China and *S. japonicum* from Mindoro, Philippines, Nei's *D* is 0.575 with one standard error of 0.220 (standard error calculated as described in Nei *et al.*, 1985). These values were used to prepare a dendrogram of genetic relatedness between the two strains (see Figure 2). This distance is slightly larger than reported by Fletcher *et al.* (1980) for the Vogel Chinese *S. japonicum* and a Philippine strain from Leyte. Comparing our results with those of Fletcher *et al.* (1980), we confirmed that 9 loci were identical in both geographic regions: *Gdh, G6pd, Hk, Ldh, Mdh-1* (anodal), *Mdh-2* (cathodal), *Me, Pep-1* (anodal), *Pep-2* (cathodal). Four newly resolved isozymes (ALD, MPI, PGD, PGI) were fixed for different alleles in the Chinese and Philippine strains. We were also able to differentiate between Chinese and Philippine *S. japonicum* at *Acp* and *Gap* as these electromorphs

were clearer than reported by Fletcher *et al.* (1980). We were unable to confirm the very slight difference at the *Pgm* locus mentioned by Fletcher *et al.* (1980), and thus believe both strains share the same PGM allele.

The lack of intrastrain genetic variability observed could not be attributed to small sample size as over 50 individuals from 40 infected snails were used;, simulations show 8-12 individuals will give a reasonable estimate of *H* (Gorman and Renzi, 1979). Alternatively, the lack of genetic variability could be due to the types of allozymes examined, which did not include nonspecific esterases and oxidases known to be highly variable. However, we were able to resolve all seven of the polymorphic loci found in comparable studies of the congeneric *S. mansoni* by Fletcher *et al.* (1981); this suggests that the loci examined were appropriate to detect protein polymorphism. One possibility, under investigation in our laboratory, is that *S. japonicum* may reproduce parthenogenically; this has been recently demonstrated by Basch and Basch (1984) for *S. mansoni*.

Until proof of asexual reproduction in these strains is obtained, we will follow all previous researchers and continue to assume that dioecious *S. japonicum* is sexually reproducing, and that Nei's *D* is an appropriate measure of genetic divergence. In order to interpret a Nei's genetic distance of 0.575 ± 0.220 between Chinese and Philippine *S. japonicum*, we may compare this value to other interstrain values reported for this species (<0.03 Fletcher *et al.*,

1981; Woodruff *et al.*, 1986a) and to genetic distances in other organisms. Ayala's (1983) survey of a wide range of organisms shows genetic distances (*D*) between conspecific populations are typically less than 0.06, and Thorpe (1983) found only 2% of conspecific population comparisons exceed *D* = 0.10. If *Schistosoma* are "typical" in their rate of amino acid substitutions and speciate with typical amounts of genetic change, the reported *D* value between the two strains of *S. japonicum* exceeds the known values for conspecific populations.

As parasite and host are thought to evolve together (e.g. Davis, 1980; Woodruff, 1985), we predict that the intermediate host snails will have diverged genetically to a similar degree. Woodruff *et al.* (1986b) reports a Nei's (1978) genetic distance between *O.h. hupensis* in the mainland of China and *O.h. quadrasi* in the Philippines, of 0.63 ± 0.20. this value is 5 times greater than the maximum genetic distance found between populations of *Oncomelania* from 4 islands in the Philippines and supports an evolutionary scenario where parasite and host diverge at similar rates.

The geographic populations of *S. japonicum* are also differentiated by intermediate host compatibility. The inability of Chinese *S. japonicum* from Shanghai to develop in *O.h. quadrasi*, the snail host from the Philippines, was experimentally shown by DeWitt (1954). Yuan *et al.* (1984) confirmed DeWitt's results and reported a markedly reduced

infectivity rate of *S. japonicum* from the Philippines in *O.h. hupensis* from China. In addition to host specificity and allozyme differences, these strains can be distinguished from one another morphologically. Sobhon *et al.* (1986) found differences in the types and distribution of papillae and the shape of the ridges on various parts of the adult body surface. These results further support the observed genetic divergence between the two strains.

The evidence indicates that "*S. japonicum*" may actually comprise a superspecies complex with several component allopatrically distributed semispecies, each of which will show some geographic variation. Such a taxonomic revision is premature, however, until comparable genetic data are available for worms from Japan, Taiwan and additional sites in China, and until all other relevant lines of evidence have been considered. Nevertheless, those working on diagnosis, treatment and control techniques (involving antibodies, vaccines and drugs) for schistosomiasis should be aware that the Chinese and Philippine strains have diverged at over 35% of their structural gene loci.

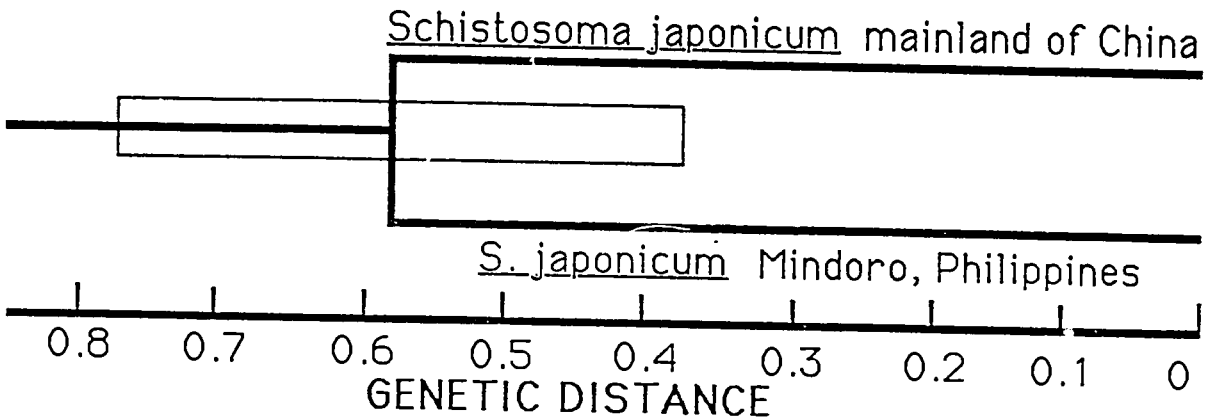

Figure 2. Phenogram based on Nei's genetic distance (1978).

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