ARTICLES

A SURVEY OF TRINIDADIAN ARTHROPODS FOR NATURAL VIRUS INFECTIONS (AUGUST, 1953 TO DECEMBER, 1958) [1]

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Arthropod investigations of the Trinidad Regional Virus Laboratory over the past five years have dealt with a variety of subjects related directly or indirectly to virology (Aitken, 1958). Throughout this period, however, primary attention has been devoted to the search for viruses in naturally infected arthropods. Accordingly, great numbers of hematophagous species have been collected annually, identified, ground up, and inoculated into white mice.

Since August, 1953, over 800 thousand specimens have thus been treated in the form of about 7,300 pools and from them 94 strains of virus have been isolated, or about one virus for every 77 pools inoculated. If one disregards 22 mosquito strains of yellow fever virus isolated during an epidemic in 1954, this figure more closely approaches one virus for every 100 pools. On the other hand, infant mice were not recipients for original insect inoculations until 19 April, 1955. As most of our mosquito agents are pathogenic for baby mice only (at least during the early passages), conceivably the yellow fever isolates might have been replaced by an equal number of baby mouse agents, and a figure somewhere between 75 to 100 pools to a virus may thus not be completely unrealistic.

One might query the advisability of continuing the annual mass inoculation of mosquitoes. Perhaps one should be more selective in the species inoculated. Actually, during the last three months of 1958 we did exercise selection in the general collections (those not taken in chicken traps), and concentrated on about a half dozen species known or thought to be vectors of Ilhéus virus and on *Culex*, a genus that seems to be particularly associated with St. Louis virus transmission. The evidence thus far gained, however, suggests that a great variety of genera and species can be found infected in the tropics, many with viruses we know little about. Moreover, the continued isolation over a period of several years of a virus such as Ilhéus from only three species among thousands inoculated, strengthens the evidence that these species are the principal vectors in Trinidad. In an exploratory program such as ours, with a bountiful supply of unknown viruses, it seems shortsighted to pattern the work along lines established by some laboratories in temperate climates where the number of viruses and vectors is limited.

In addition to the general sampling of the arthropod population in Trinidad, the entomologist has frequently been called upon to set up special collection programs in and around houses of patients from whom a virus was isolated. There was a considerable amount of such activity in 1954 in connection with yellow fever investigations; numerous attempts were made that year to isolate yellow fever virus from *Aedes aegypti* and other mosquitoes col-

[1] The studies and observations on which this paper is based were conducted with the support, and under the auspices, of the Government of Trinidad and Tobago, the Colonial Development and Welfare Scheme, and The Rockefeller Foundation.

Presented at the 15th Annual Meeting of the American Mosquito Control Association, 13–15 April, 1959, Salt Lake City, Utah.

lected in houses of suspect fever cases—none of them, however, successful.

COLLECTING AND TRANSPORTING INSECTS. The following remarks apply mainly to adult mosquitoes since these constitute our principal study material.

Mosquitoes are caught with plastic aspirator tubes and blown into pint ARC-LID Mason jars fitted with inverted perforated plastic cones in the screw-cap lid. These jars are lined with plaster of paris, which provides the mosquitoes with humidity (when it is moistened) as well as with a better surface on which to cling. The cone lids are plugged with a bit of cotton to prevent any escapes and during transit to the laboratory the jar tops are covered with a square of cloth held in place with rubber bands. No attempt is made to ice the live insect catch until it is ready for transportation to the lab. Ordinarily not more than 200 mosquitoes are put in one jar, but on occasion, when densities are high and jars scarce, there have been as many as 400 per jar.

Heleids (*Culicoides*) and other small Diptera are likewise collected by aspiration and kept in the same jars with the mosquitoes. This is admittedly not good practice, since the *Culicoides* in particular may escape through the perforations of the cone, but thus far we have hesitated to provide our mosquito catchers with an additional special bottle for small Diptera. Horse flies are caught with small hand nets and lightly anesthetized in a chloroform killing tube. They are then transferred to shell vials along with a paper giving the hour and circumstances of the capture, and stored on ice as captured.

The jars and vials of insects are transported to the Port-of-Spain laboratory by taxi (at times by train) in portable aluminum ice boxes equipped with a rack to hold the jars upright. On the occasional day trip, mosquito jars are transported in Koroseal-covered, zippered "Thermo-Keep" insulated bags, 16 by 8 by 10 inches deep; the refrigerant consists of "Sno-Gel" No. 26 bricks which, when not in use, are stored in a deep freeze box. Thus far we have not had to transport routine collections more than 30 to 40 miles. The collecting period ordinarily being during the day (e.g. 7 a.m. to 3 p.m.), the mosquitoes arrive at the lab in the late afternoon. Formerly stored overnight at 4° C., they are now kept at room temperature in order to permit any engorged mosquitoes more time to digest their blood meals.

The following morning, the mosquitoes are "knocked down" with tobacco smoke and in about 15 minutes are ready for examination. (Jars formerly kept at 4° C. were first brought into a dry room, where they were kept covered securely to prevent water of condensation forming on the inside of the jar, and allowed to warm up for about 20 minutes.)

PROCESSING ARTHROPODS FOR VIRUS ISOLATION. Following anesthetization, a jar of insects is emptied into a Petri dish and the contents identified. The individual species are sorted into tubes held by a rack in an ice bath. The tubes used for this purpose are old 30 ml "Vacune" tubes (7/8-inch diameter) cut to a length of 4 inches. To prevent the insects' sticking to the glass, the tubes are lined with 3- by 5-inch sheets of weighing paper, which are wrapped around a wooden plunger for insertion into the tube. Each tube carries a recognition "flag," an applicator stick with the species name typed on a cardboard indicator. As an aid to virus survival, an attempt is made to keep the insects cold at all times except when under a microscope. A routine morning's operation may require the identification of a few hundred to several thousand mosquitoes representing 40 to 50 species.

Ordinarily it is not possible to grind and inoculate a catch the same day it is identified. Therefore, the species are stored as whole insects at —60° C. either in sealed glass ampoules in a dry ice box, or in an electric box (Revco) in open tubes which permit accumulation of additional material. Formerly engorged mosquitoes were discarded, but as they are now stored overnight at ambient temperatures, engorged and unengorged specimens are treated similarly—except that if particularly plentiful, as in the case of chicken-

trap collections, the engorged mosquitoes may be ground separately.

Ampoules of stored insects are held until sufficient numbers have accumulated and mice are available. Insects may thus be stored for a day or so up to a month. Most species are ground about twice a month, but in the season of greatest prevalence a few of the most abundant species are ground several times during the month. To make record keeping easier, stored insects are ordinarily not carried over the month.

Mosquito pools are suspended in diluent according to the following schedule: 1 ml for the first 10 mosquitoes; 2 ml for 11 to 300 mosquitoes; and 3 ml for more than 300 mosquitoes.[2] Occasionally, large pools of 700 or more specimens are broken down into two pools.

Certain mosquitoes (and ticks) are toxic to 2-day-old mice when inoculated intracerebrally (i.c.) in large numbers. In order to overcome the problem of retreating the specimen and reinoculating, during the past year a modification of the dilution schedule for certain species (e.g. *Aedes scapularis, A. serratus, Psorophora ferox,* and *Culex fatigans*) was instituted, as indicated below:

No. of mosquitoes	Original suspension (ml)	Subsequent dilution factor
300–400	3	1:2
400–600	3–4	1:3
600–1,500	4–9	1:4
1,500–2,000	9–10	1:5
2,000 plus	10	1:6

By the dilution factor, we mean that following centrifugation, the suspension is further diluted 1:2, etc. The original suspension, however, is stored for future reference.

In the case of *Psorophora ferox* and *Culex fatigans,* the 1:2 dilution factor was subsequently applied to pools of 150 to 400 mosquitoes. It has been noted that a pool of about 150 chick-blooded *Culex taeniopus* or *C. spissipes* should likewise be diluted 1:2, even though these are small species. We have been forced to resort to these measures because of the large numbers of mosquitoes confronting us at certain times of the year. While some of the pools have of necessity been large, generally they run between 20 and 50 specimens per pool. In one instance, however, we isolated Ilhéus virus from a pool of 2,055 *Aedes serratus* suspended in 6 ml diluent and further diluted 1:6, and there have been many isolations from pools of 200 to 700 mosquitoes.

With respect to other arthropod suspensions, we have adopted roughly the following schedule (trying to keep diluent to a minimum at all times):

Phlebotomus, simuliids, Anoplura, Mallophaga—100 per 1 ml.

Culicoides and mites—500 per 1 ml.

Tabanids—2 ml for 1 to 10 flies, thereafter 1 ml for every 10 flies.

Philornis—1 to 4 flies in 1 ml, 5 to 20 in 2 ml, 20+ in 3 ml.

Ticks—These are more difficult to handle because they are laden with bacteria and fungi, particularly if kept alive in small tubes for some time. Unengorged nymphs, about 100 per 1 ml; engorged nymphs 5 per ml; males up to 20 in 1 ml and engorged females about 1 to 2 ml per tick. Ticks engorged with cattle blood are toxic. Since filtration is useless, specimens must be diluted. Ticks have also been successfully inoculated after being cleansed in 70 percent alcohol.

Following grinding, suspensions are centrifuged at 8,000 r.p.m. for 1/2 hour at 4° C. Subsequently 2-day-old mice receive an inoculum of 0.02 ml i.c. and the remainder is stored at —60° C. Mice are held 21 days for signs of illness before be-

[2] The diluent in which mosquitoes are triturated is composed of 0.12 molar NaCl; 0.006 molar NaH_2PO_4; 0.012 molar $NaHPO_4$; and 0.75 percent fraction 5 bovalbumin. The pH is adjusted to between 6.8 and 7.3 by the addition of 0.3 molar NaOH solution. Sterilization is effected by filtration through a Seitz-EK pad. Crystalline penicillin G and streptomycin sulphate are added to produce a final concentration of 400 units per ml of the former and 250 μg per ml of the latter.

TABLE 1.—Trinidadian mosquitoes inoculated into adult and baby mice, 1953–1958

Species	No. mosquitoes inoculated into mice		Species	No. mosquitoes inoculated into mice	
	Adult	Baby		Adult	Baby
Aedes aegypti	25	3	*Limatus durhami*	1,546	9,770
fulvithorax	258	468	*flavisetosus*	3,767	27,287
fulvus	56	1,741	*Mansonia arribalzagai*	173	50,116
hortator	36	1,613	*titillans* *	694	2,134
ioliota	72		*venezuelensis*	2,224	8,487
leucocelaenus	253	18	*Orthopodomyia fascipes*		4
scapularis	580	46,592	*Phoniomyia fuscipes* (?)	1,068	15
serratus	2,046	115,621	*lassalli*	1	17,278
sexlineatus	9,995	866	*splendida*	1,127	3,291
taeniorhynchus	3	833	*trinidadensis*	4,814	24,440
terrens	161	3	*Psorophora albipes*		58,216
sp.	140		*cingulata*	13,636	3,722
Anopheles apicimacula	335	1	*ferox*	4,380	61,879
aquasalis	20,900	3,863	*lineata*		1
bellator	16,818	5,302	*lutzi*	321	2,595
eiseni	20		sp.	105	
homunculus	14,880	503	*Sabethes belisarioi*		9
mediopunctatus	168	3	*chloropterus*	191	858
neomaculipalpus	44	21	*cyaneus*	1	123
nimbus	11	276	*undosus*	1,665	201
oswaldoi	258	332	*Trichoprosopon digitatum*	147	956
sp.		46	*longipes*	2,295	914
Culex caudelli		7,812	*theobaldi*	13,389	2,306
corniger		1	*Wyeomyia aporonoma*	11,354	2,019
coronator		480	*arthrostigma*	1	1,715
gairus		5	*autocratica*		508
gaudeator		6	*circumcincta* (?)		21
mollis		1	*clasoleuca* (?)		895
nigripalpus	12	3,768	*felicia*	26	316
quinquefasciatus	201	9,660	*finlayi*		2,510
spissipes	365	5,323	*hemisagnosta* (?)		22
stonei		3	*howardi*	3	13,588
taeniopus	56	1,752	*kerri*	3	107
urichi	1	42	*medioalbipes*	2,296	6,895
virgultus		251	*melanocephala*	1	2,445
sp. #2		481	*melanopus* (?)		205
sp. #3		286	*personata* (?)		1
sp. #7		212	*pseudopecten*	1	1,293
sp. #8		971	*scotinomus*	13,326	411
sp. #9		1	*ulocoma*		13
sp. #10		740	*ypsipola*		5,259
sp. #11		163	sp. near *serrata*	143	
sp. #12		35	sp.	134,831	18,752
sp. #13		144			
sp.	2,524	2,242	Total	289,373	553,208
Haemagogus spegazzinii	5,686	9,147			

* Species previously referred to by us as *Mansonia wilsoni*.

ing discarded. Specimens in litters with 4/7 mortality ratio or higher are reinoculated.

ARTHROPODS SURVEYED. In the five years and 4-1/2 months during which Trinidadian arthropods have been processed in the search for natural virus, 842,581 mosquitoes and 13,262 other arthropods have been inoculated into adult and baby mice—a grand total of 855,843 arthropods processed. Adult mice were the recipients of 290,804 arthropods and infant mice of 565,039 arthropods.

The 84 species of mosquitoes and the numbers of each inoculated into the two types of mice are presented in Table 1. The six most commonly tested species are (approximate figures): *Aedes serratus* (118,000), *Psorophora ferox* (66,000), *Psorophora albipes* (58,000), *Mansonia arribalzagai* (50,000), *Aedes scapularis* (47,000), and *Limatus flavisetosus* (31,000).

Inoculations are presented on a generic basis in Table 2 and Fig. 1. It will be noted that *Wyeomyia* is the genus most commonly inoculated. Almost three quarters of these Wyeomyias were handled during a period early in the history of the laboratory when we were not differentiating species in this rather difficult genus either through lack of time or because of unfamiliarity with the group. In 1953–54 and early in 1955, when only adult mice were inoculated with original mosquito material, the principal genera inoculated were *Wyeomyia, Anopheles, Psorophora, Trichoprosopon,* and *Aedes.* Much of the work in those days was in mountainous districts and mangrove swamps. Since the change-over to suckling mice in April, 1955, the scene of activities has changed to lowland swamp-forest and cocoa and citrus plantations, characterized largely by ground pool and permanent water breeding species. As a result *Aedes* and *Psorophora* have become the dominant genera encountered, followed by *Mansonia* and the ubiquitous Wyeomyias. In this latter period there has also been a noteworthy increase in the collections of *Phoniomyia, Limatus,* and *Culex.*

In the non-mosquito groups (Table 3), little testing was done until the past few years. *Culicoides* and simuliids have received the greatest attention. Obviously more work needs to be done with these and related groups before it can be said that they have been adequately sampled. The chalcids were included because they were parasitizing *Philornis* pupae, which in turn came from larvae parasitic on a nestling silver-beak tanager from which St. Louis virus was isolated. The genus *Philornis* represents a group of flies most of which are parasitic on fledgling birds in the Neotropical Region. They have been shown to constitute a highly

TABLE 2.—Trinidadian mosquitoes, by genera, inoculated into adult and baby mice (1953–1958)

	Adult mice	Baby mice	Grand total
Aedes	13,625	167,758	181,383
Anopheles	53,434	10,347	63,781
Culex	3,159	34,379	37,538
Haemagogus	5,686	9,147	14,833
Limatus	5,313	37,057	42,370
Mansonia	3,091	60,737	63,828
Orthopodomyia		4	4
Phoniomyia	6,950	45,024	51,974
Psorophora	18,442	126,413	144,855
Sabethes	1,857	1,191	3,048
Trichoprosopon	15,831	4,176	20,007
Wyeomyia	161,985	56,975	218,960
Total	289,373	553,208	842,581

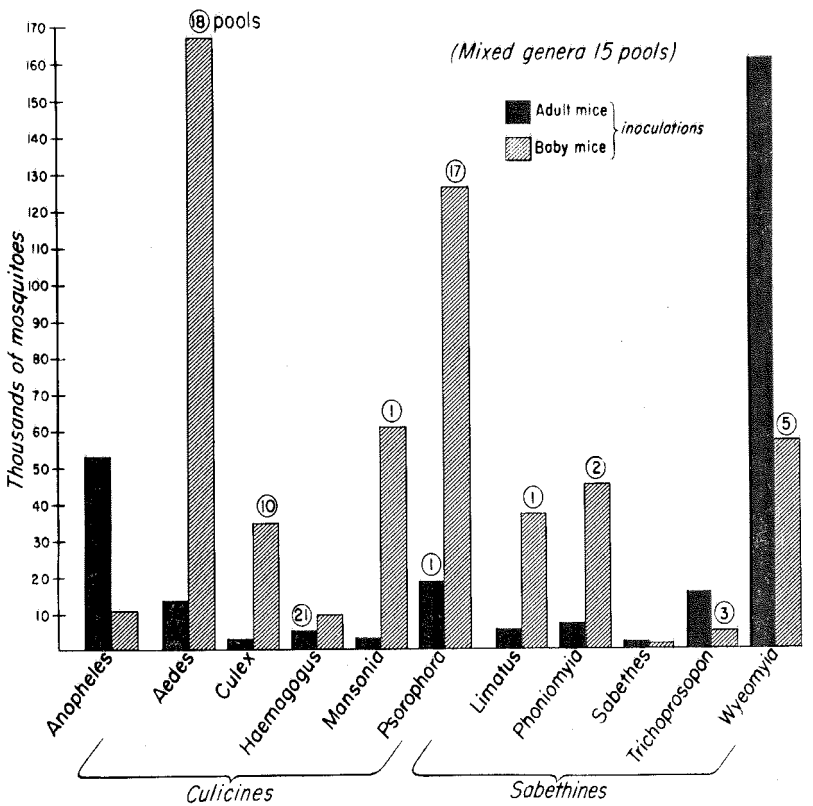

FIG. 1.

favorable medium for virus growth (Aitken, *et al.*, 1958), and because of their hematophagous habits could conceivably occupy a niche in some virus transmission cycle. Arthropod inoculations by group and year are summarized in Table 4.

VIRUSES ISOLATED. Since 1953, a total of 94 viruses have been isolated from arthropods—all from mosquitoes. As shown in Table 5, these have been classified into 15 distinct agents on the basis of immunological studies carried out in this laboratory; included in the table are the species of mosquitoes from which the strains were isolated as well as the number of strains obtained from each. Table 6 summarizes virus isolations by genus of mosquito.

Twenty-four of these viruses were isolated in 1954 from adult mice. Of this number, 22 represented strains of yellow fever virus (Downs *et al.*, 1955) and two were strains of Ilhéus virus (Anderson *et al.*, 1956).

All of the remaining 70 strains, representing 14 agents, have been isolated in infant mice since April, 1955. Three of these agents are previously known viruses —Ilhéus, St. Louis (Anderson, Aitken *et al.*, 1957), and Mayaro (Anderson, Downs *et al.*, 1957)—but most of the other 11

TABLE 3.—Trinidadian arthropods other than mosquitoes inoculated into adult and baby mice, 1953–1958

Species	No. arthropods inoculated into mice: Adult	Baby
Simuliids		
Simulium incrustatum	22	1,170
metallicum	62	54
samboni	343	2,014
Total	427	3,238
Heleids		
Culicoides debilipalpis	0	13
diabolicus	0	5,315
sp.	0	1,180
Total	0	6,508
Psychodids		
Phlebotomus sp.	952	20
Tabanids		
Acanthocera sp.	0	84
Chlorotabanus inanis	0	2
mexicanus	0	2
unicolor	0	16
Chrysops variegata	0	269
Leucotabanus exaestuans	0	87
Stibasoma fulvohirtum	0	139
mallophoroides	0	54
Tabanus colombensis	0	7
lineola var. *carneus*	0	364
sorbillans	0	54
xipe	0	86
Total	0	1,164
Anthomyiids		
Philornis sp. (several)	0	346
Chalcids		
Spilochalcis sp.	0	2
Mallophagans		
Chicken lice	0	161
Ticks		
Amblyomma cajennense	0	111
dissimile	0	3
longirostre	0	8
Boophilus microplus	47	173
Rhipicephalus sanguineus	0	47
Total	47	342
Mites		
Dermanyssids	5	50
Grand Total	1,431	11,831
		13,262

TABLE 4.—Trinidadian arthropods, by years, ground and inoculated into mice (August, 1953–31 December, 1958)

Arthropods	1953	1954	1955	1956	1957	1958	Total
Mosquitoes	10,126	278,866	154,861	76,549	151,155	171,024	842,581
Culicoides	0	0	1,497	65	367	4,579	6,508
Simuliids	0	427	379	775	1,162	922	3,665
Tabanids	0	0	0	0	509	655	1,164
Phlebotomus	0	952	20	0	0	0	972
Ticks	0	47	0	0	0	342	389
Philornis	0	0	0	0	0	346	346
Lice	0	61	100	0	0	0	161
Mites	0	0	5	0	0	50	55
Chalcids	0	0	0	0	0	2	2
Total	10,126	280,353	156,862	77,389	153,193	177,920	855,843

Note: All arthropods in 1953 and 1954, and 381 mosquitoes in 1955 inoculated into adult mice; remainder inoculated into baby mice.

TABLE 5.—Preliminary classification of viruses isolated from Trinidadian mosquitoes, 1953–1958

Classification Prototype virus	Total # strains	Collection site	*Source of virus* Mosquito species	No. strains by mosq. sp.
Mayaro TR 15537	1	Rio Grande Forest	*Mansonia venezuelensis*	1
Yellow fever TR 2942	22	Melajo Forest	*Haemagogus spegazzinii*	8
			Mixed pool	1
		Charuma Forest	*Haemagogus spegazzinii*	13
St. Louis TR 9464	6	Melajo Forest	*Culex caudelli*	1
			Psorophora ferox	1
		Arena Forest	*Culex coronator*	1
		Vega de Oropouche	*Culex coronator*	1
			Culex spissipes	1
			Culex taeniopus	1
Ilhéus 3089 (Brazilian strain)	18	Melajo Forest	Mixed pool	1
		Charuma Forest	*Psorophora* pool	1
		Rio Grande Forest	*Psorophora ferox*	6
			Aedes serratus	3
			Aedes scapularis	2
			Psorophora pool	1
			Mixed pool	3
		Vega de Oropouche	*Culex caudelli*	1
TR 7994	1	Arena Forest	*Trichoprosopon* pool	1
TR 8349	17	Rio Grande Forest	Mixed pool	9
			Aedes scapularis	2
		Melajo Forest	*Psorophora albipes*	1
			Limatus pool	1
			Aedes scapularis	2
			Trichoprosopon longipes	1
		Vega de Oropouche	*Psorophora* pool	1
TR 8362	1	Melajo Forest	*Culex* pool	1
TR 8762	4	Melajo Forest	*Psorophora albipes*	2
			Psorophora ferox	2
TR 8900	11	Melajo Forest	*Aedes scapularis*	4
			Psorophora ferox	2
			Wyeomyia pool	2
			Wyeomyia aporonoma	1
			Culex spissipes	1
		Rio Claro	*Wyeomyia ypsipola*	1
TR 9223	6	Melajo Forest	*Trichoprosopon theobaldi*	1
			Wyeomyia pool	1
		Rio Grande Forest	*Phoniomyia* pool	2
			Psorophora ferox	1
		Vega de Oropouche	*Culex sp.* #7	1
TR 9375	1	Melajo Forest	*Aedes scapularis*	1
TR 10076	3	Melajo Forest	*Aedes scapularis*	2
		Rio Grande Forest	*Aedes scapularis*	1
TR 11573 (ex Artibeus)	1	Rio Grande Forest	Mixed pool	1
TR 18462	1	Rio Claro	*Culex sp.* #8	1
TR 20659	1	Rio Grande Forest	*Aedes scapularis*	1
Total	94			94

TABLE 6.—Virus isolations in Trinidad according to arthropod source (1953–1958)

Mosquito genus	Virus															
	YF	Ilhéus	SL	Mayaro	7994	8349	8362	8762	8900	9223	9375	10076	11573	18462	20659	Total
Culex		1	5				1		1	1				1		10
Mansonia				1												1
Aedes		5				4			4		1	3			1	18
Psorophora		8	1			2		4	2	1						18
Haemagogus	21															21
Trichoprosopon					1	1				1						3
Wyeomyia									4	1						5
Phoniomyia										2						2
Limatus						1										1
Mixed	1	4				9							1			15
Total	22	18	6	1	1	17	1	4	11	6	1	3	1	1	1	94

appear to be new to science, although some show relationships to viruses isolated in other parts of the Western Hemisphere.[8]

A number of mosquito genera are included in the list of suspect vector species (Fig. 1): *Mansonia, Culex, Aedes, Psorophora, Trichoprosopon, Limatus, Wyeomyia,* and *Phoniomyia.* The species most frequently found infected are *Aedes scapularis, Psorophora ferox,* and *P. albipes.* It is unfortunate that mixed pools were the source of virus in several instances; these were unavoidable happenings at times when few mice were available for virus isolation attempts.

SUMMARY. Techniques are reported for the processing of Trinidadian arthropods for recovery of naturally occurring virus. Mosquitoes have been the principal group investigated. Tables are presented listing the numbers of the various species inoculated into adult and baby mice and the numbers and kinds of viruses isolated from them during the period August, 1953 to December, 1958.

[8] One of these, TR 8900, has now been named Kairi virus (Anderson *et al.,* 1960) and has been shown to be related to Wyeomyia and Bunyamwera viruses (Casals and Whitman, 1960).

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