

**EUTELY IN WUCHERERIA BANCROFTI MICROFILARIAE  
(NEMATODA: FILARIOIDEA) FROM DIFFERENT  
GEOGRAPHIC AREAS IN RELATION TO ITS  
EVOLUTION AND DISPERSAL**

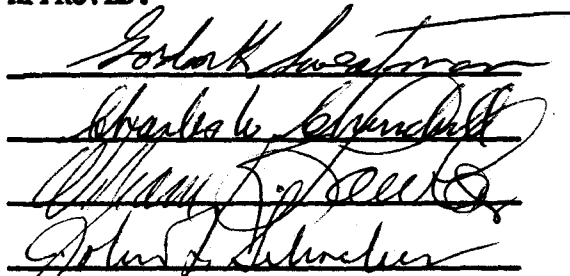
**A Thesis**

**by**

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**EUTELY IN**  
**WUCHERERIA BANCROFTI**

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## ABSTRACT

Microfilariae of Wuchereria bancrofti from fifteen different geographic areas were examined according to the concepts of eutely. Blood films were stained in Delafield's hematoxylin following fixation in hot (60C) ethanol (Fulleborn, 1924). The number of nuclei in the region between the cephalic space and nerve ring, and between the nerve ring and excretory pore of fifty microfilariae from each geographic region were counted. Calculations of means, standard deviations, standard errors and coefficients of variation were made; cross-matched data were analyzed using the t-test. Nuclear numbers in four Indian and a Ceylonese isolate coincided almost exactly, and were very close to those found in material from Egypt. Isolates from Brazil and Singapore(A) were quite distinct from each other and the other strains; the remainder of the nocturnally periodic material appeared to form a cline of intergrading forms. Morphological differences between microfilariae combined with strong biological differences such as periodicity support the establishment of Wuchereria bancrofti pacifica (Manson-Bahr and Muggleton, 1952). A new sub-specific taxon may possibly be erected for Brazilian material. Wuchereria vauceli Galliard, 1959 is suppressed. Aspects of the evolution of Wuchereria bancrofti are discussed; the possible use of this parasite in anthropological surveys is pointed out.

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## INTRODUCTION

Microfilariae of Wuchereria bancrofti (Cobbold, 1877, Seurat, 1921) (Nematoda: Filarioidea) were first seen by Demarquay (1863, in Manson-Bahr, 1959) in lymphocele fluid from a Cuban patient residing in Paris. Subsequently, Wucherer (1866, in Manson-Bahr, l.c.) recovered them from the urine of patients in Salvador, Brazil. Lewis, working in India, first described microfilariae in blood (1872, in Iyengar, 1957), calling them Filaria sanguinis hominis. Adults recovered by Bancroft (1876 - 1877, in Manson-Bahr, l.c.) from a Chinese patient in Queensland, Australia, were studied by Cobbold (1877, in Faust, 1949) who gave them the name Filaria bancrofti. At about the same time, Lewis (1877 in Iyengar l.c.) reported the recovery of adults in an Indian patient, but did not pursue the matter taxonomically.

Since that time, this parasite (with several biologically distinct varieties) has been shown to have a wide distribution in tropical and subtropical regions of the world (Text fig. 1). The only vertebrate host thus far demonstrated is man; and the few attempts thus far made to transmit this parasite to other animals have been unsuccessful (Laing et al., 1960; Ramachandran et al., 1966).

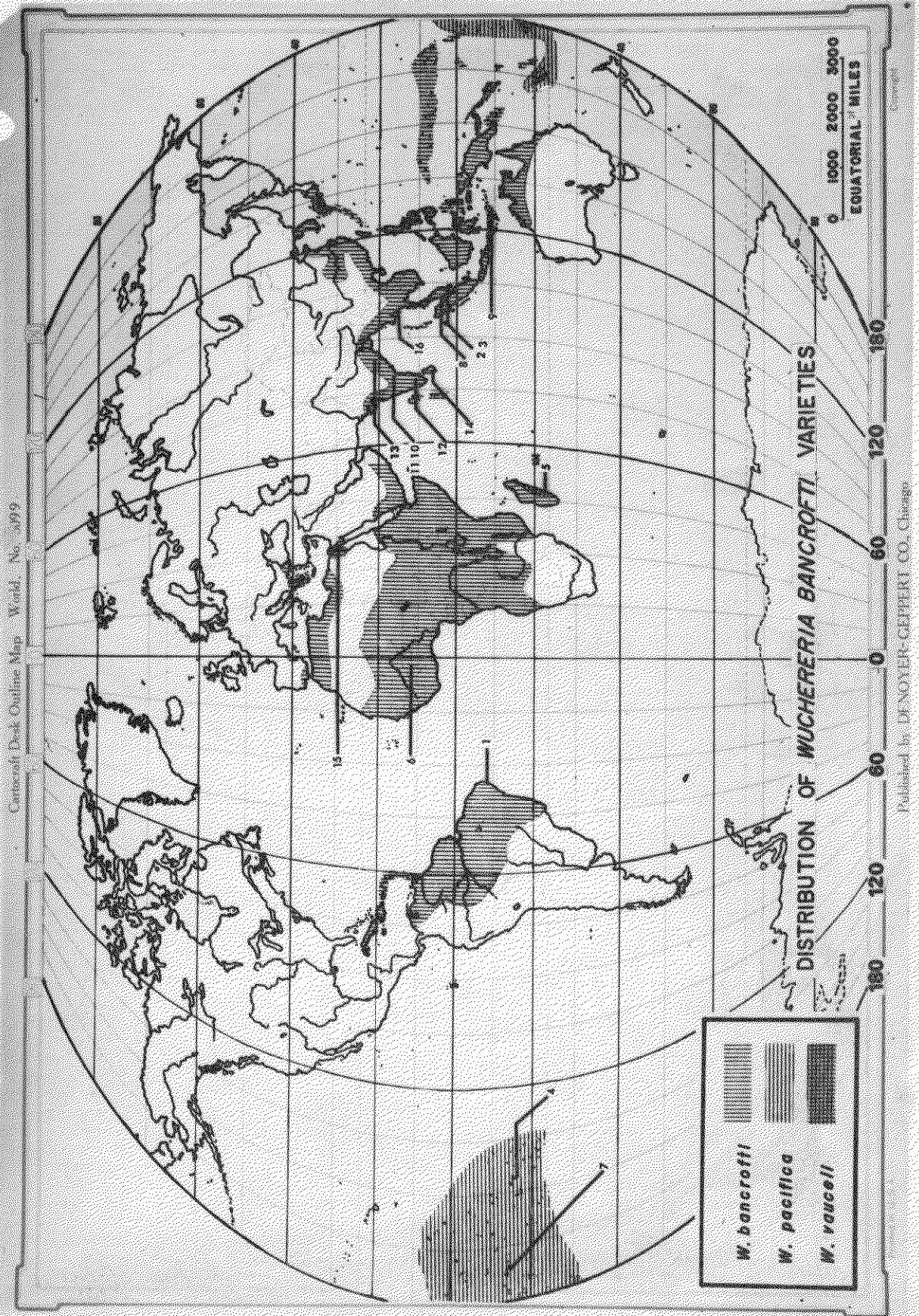
A vast variety of both culicine and anopheline mosquitoes have been incriminated as vectors of Wuchereria bancrofti (de Rook, 1957: in Manson-Bahr, l.c.). Microfilariae which are ingested with the blood undergo metamorphosis within the thoracic muscles of the arthropod and infective forms (third-stage larvae). deposited on the skin of the final host at the time of feeding, probably penetrate through the bite-wound (Ewert, 1967) and migrate to their final place of maturation and development in the lymphatics (Schacher and Sahyoun, 1967).



TEXT FIGURE I

The Geographic Distribution of Wuchereria bancrofti and its Major Biological Varieties (redrawn, mostly after Faust and Russell, 1964). Numbers Indicate the Geographic Distribution of Material Examined in the Present Study and Correspond to Those in Table I.

TEXT FIGURE 1.



Wuchereria bancrofti is disturbingly inconstant in different geographic areas with respect to biological properties such as periodicity, vector, pathogenesis and occasionally, the reaction of infected patients to specific antifilarial therapy. Attempts have been made on these and other grounds to erect sub-species or varieties such as Wuchereria bancrofti var. pacifica (Manson-Bahr and Muggleton, 1952) and Wuchereria bancrofti var. vauceli (Galliard and Brygoo, 1955), the latter subsequently raised to species status (Galliard, 1959). Although minor morphological differences have been found between adult worms from different areas (Fain, 1951; Buckley, 1952; Buckley and Singh, 1965), biometric studies have been hampered by inadequate adult material and the taxonomic status of both varieties is in doubt.

As microfilariae are the only easily obtainable stage from man, they have had justifiably time-honored use in diagnosis at the species level. For this, their "attitude" in blood films, staining reaction, body and cephalic space dimensions, the relative placement of the nerve ring, excretory pore, excretory cell and "R" cells combined with the extent of caudal nucleation have sufficed. However, except for Wuchereria bancrofti var. vauceli, these characters have not been used at the infra-specific level.

Although many mosquito species have been reported capable of supporting growth of larvae of Wuchereria bancrofti to the third stage, the capability of a given intermediate host is genetically based (Macdonald, 1956; Wharton, 1960). Macdonald suggests that "differences in gene frequencies may account for the cases where different populations of species differ in response to a single strain of a parasite. Similarly, differences in the parasites may be the reason for variation among different strains".

As the species concept in parasitic helminths derives primarily from morphological data (although the concept of a "biological species" seems to be gaining strength; Mayr, 1964), we have felt justified in examining microfilariae according to the concepts of nuclear constancy or eutely

(De Coninck, 1938). Initially shown by Loos (1896) in ascarids, this theory in essence states that size increase in eutelic forms is by cell growth rather than multiplication, and that number, relative size, form and distribution of cells are constant. Martini (1923), De Coninck (1938), and Nigon (1965) have reviewed this widely acknowledged phenomenon in several invertebrate groups including Nematoda.

To avoid being filtered out of the circulation, microfilariae cannot afford growth in the vertebrate host, and probably will not grow in the absence of specific (unknown) physiological stimuli and growth factors found in the arthropod. Therefore, nuclear multiplication appears either to be precluded or to be restricted to a minimum once the microfilaria is released from the parent worm. Indirect evidence such as the susceptibility of early developing forms to organic arsenicals while mature microfilariae are not affected (Friedheim, 1966) seems to further justify the belief that microfilariae should be a stable stage, both physiologically and morphologically, and that any individual variations in either aspect might be tested by statistical analysis.

Discussions of the evolution of filarial worms have in general tended to center around the evolution of different groups of definitive hosts and the mode of feeding (and its evolutionary consequences) of arthropod intermediate hosts (Anderson, 1958; Chabaud, 1954; Lavoipierre, 1958; Hawking and Worms, 1961; Nelson, 1964). In relation to Wuchereria bancrofti, the only meaningful discussions regarding its evolution have been those related to the possible derivation of sub-periodic from periodic forms (Iyengar, 1965).

The purpose of the present study is to relate differences in nuclear numbers in microfilariae from different geographic areas with possible taxonomic differences between isolates and the evolution of this species.

As man is the only vertebrate host, and geographic dispersal of the worm is primarily if not solely by man, the possibility that interrelationships between people of various regions might be demonstrable by analysis of the "strains" of worms harbored by them will be assessed.

## MATERIALS AND METHODS

Dehemoglobinized and dried but unfixed thick blood films containing microfilariae of Wuchereria bancrofti were obtained from collaborators working in various geographic regions (Table 1.; Fig. 1).

Blood films were fixed in 70% ethanol at 60C for 30 minutes, stained in Delafield's hematoxylin, differentiated briefly, dehydrated in an alcohol series, cleared in xylol and mounted in balsam (Fulleborn, 1924). Counts were then made under high magnification of the number of nuclei in the region between the cephalic space and nerve ring, and between the nerve ring and excretory pore of microfilariae. Nuclei were counted in only those microfilariae which were reasonably straight and in which the nerve ring and excretory pore were recognizable. Nuclei of at least fifty microfilariae from each geographic area mentioned were counted three times, and all the slides from a given geographic area were handled and stained similarly and simultaneously. The number of patients examined, their sex and racial group are shown in Table 2.

Calculations of means, standard deviations, standard errors and coefficient of variations and analysis of cross-matched data were facilitated by use of an IBM 1620<sup>R</sup> Computer.

TABLE 1

PERIODICITY AND NATURAL VECTORS OF ISOLATES OF  
WUCHERERIA BANCROFTI MICROFILARIAE STUDIED FROM  
VARIOUS GEOGRAPHIC AREAS

Origin of material	Periodicity	Vector	Authority*
1- Brazil	Nocturnally periodic	<u>Culex p. fatigans</u>	(Rachou, 1956)
2- Singapore	Nocturnally periodic	<u>Culex p. fatigans</u>	(Danaraj <u>et al.</u> , 1958)
3- Tahiti	Diurnally sub-periodic	<u>Aedes polynesienses</u> <u>Aedes samoanus</u> <u>Aedes upolensis</u>	*
4- Madagascar	Nocturnally periodic	<u>Anopheles coustani</u> <u>Mansonia uniformis</u>	(Schacher, 1967)
5- Upper volta	Nocturnally periodic	<u>Anopheles gambiense</u> <u>Anopheles funestus</u>	*
6- Western Samoa	Diurnally sub-periodic	<u>Aedes polynesienses</u> <u>Aedes samoanus</u> <u>Aedes upolensis</u>	*
7- Selangor	Nocturnally periodic	<u>Anopheles letifer</u>	(Wharton, 1960)
8- Timor	Nocturnally periodic	Unknown	(David, H.L. and Edeson, 1965).
<u>India:</u>			
9- Rajahmundry )	Nocturnally periodic	<u>Culex p. fatigans</u>	*
10- Rayavaram )			
11- Calicut )			
12- Varanasi )			
13- Ceylon	Nocturnally periodic	<u>Culex p. fatigans</u>	*
14- Egypt	Nocturnally periodic	<u>Culex p. complex</u>	*
15- Thailand	Nocturnally sub-periodic	<u>Aedes rivens</u>	*

\* Unless otherwise denoted, the authority for this information is the personal communication of collaborators collecting and sending material.

TABLE 2.

SEX, ETHNIC GROUP AND NUMBER OF PEOPLE EXAMINED FROM  
DIFFERENT GEOGRAPHIC AREAS

Origin of material	Male	Number of people		Total	Ethnic group
		Female	Unrecorded		
Brazil			3	3	Mixed
Singapore	14	2	15	31	Indian, Chinese, Malay
Tahiti	5			5	Polynesian
Madagascar	7			7	Malagache
Upper Volta	5			5	Negro
Western Samoa	2	1		3	Polynesian
Selangor	5			5	Aborigine (SEMAI)
Timor	1	1	1	3	Melanesian
India:					
Rajahmundry )					
Rayavaram )	12	4		16	Indian
Calicut )					
Varanasi )					
Ceylon	7			7	Sinhalese
Egypt	3			3	Egyptian
Thailand			2	2	Thai



## PRESENTATION OF DATA

### I. Nuclear number in Microfilariae

The mean, standard error, standard deviation and range in number of nuclei between the cephalic space, nerve ring and excretory pore of microfilariae from different geographic areas are shown in Tables 3 and 4. Table 5 presents the total number of nuclei found between the cephalic space and excretory pore (i.e., a summation of Table 3 and 4). Graphic presentation of this information is given as population range diagrams in text figures 4 and 5, affording visual comparison of data included in the tables. Tables 3 to 5 also show coefficients of variation for each measurement to test homogeneity and adequacy of the sample (numbers much below 4 indicate an inadequate sample to show the real variability; much above 10, that the sample was not pure; (Hansen et al. 1953; Simpson et al. 1960.)

### II. Statistical analysis of data

Essentially, three groups of data were available for analysis and comparison of microfilariae from each geographic area: (1) The number of nuclei between the cephalic space ( CS ) and nerve ring (NR), (2) the number between the latter and the excretory pore (EP), (3) the sum-total of these (CS-EP).

#### A. Nuclei between CS-NR.

Considerable variation was found in both the range and the mean number of CS-NR nuclei. The lowest numbers in both range and mean were found in material from Brazil, the highest in material from Thailand. (Table 3, Text figure 3). An essentially normal curve distribution of CS-NR nuclear counts was seen in frequency diagrams constructed for data from all isolates except that from Singapore, where a bimodal distribution was found (Text figure 3).

A "goodness of fit" test of data from Singapore indicated that it could not be fitted to a single normal curve, and therefore did not represent a single universe ( $\chi^2 = 31.659$ ;  $P = 0.0005$ ). These microfilariae were therefore segregated into two separate groups designated as Singapore A and B.

Analysis of 360 reciprocally cross-matched groups by the t-test showed highly significant differences between all groupings except those shown in table 6.

#### B. NR-EP number

Again, considerable variation in range and mean was seen between data of the various isolates (Table 4; Text Fig. 4). The lowest numbers both in range and mean were again in material from Brazil, the highest in Thailand.

Analysis of cross-matched NR-EP numbers by the t-test showed highly significant differences except in those matchings outlined in table 7.

#### C. CS-EP number

Statistical analysis of the total number of nuclei between the cephalic space and excretory pore (Table 5, Text fig. 6) was again performed by t-test and showed a high order of separation between all cross-matches of isolates except those outlined in table 8.

As can be seen in tables 6-8, isolates were statistically distinct or not to varying degrees. Some were statistically distinct in cross-matchings in CS-NR, NR-EP and in the total CS-EP number; others were distinct in either one or two measurements, but could not be separated on the bases of the third. For this reason, a bidirectional graph was prepared comparing simultaneously the CS-NR and NR-EP nuclear numbers of isolates of "periodic" microfilariae (Text fig. 6) and "sub-periodic" microfilariae (Text fig. 7). The first of these shows that the isolates from Brazil and Singapore A and (to lesser degree) Thailand and Singapore B appear more or less distinct. Material from

all regions in India and Ceylon coincided to such a marked degree that only one indication could be given on the graph.

TABLE 3

Range, Mean, Standard Deviation, Standard Error and Coefficient of Variation in the Number of Nuclei Between the Cephalic Space and Nerve Ring of 50 - 78 Microfilariae of Wuchereria bancrofti from Different Geographic Regions.

Origin of material	Number	Range	Mean	S.D.	S.E.	V.
Brazil (Urban)	50	58-72	64.40	3.92	.554	6.08
Singapore A. (Urban)	75	58-72	65.76	3.49	.403	5.30
Singapore B. (Urban)	78	73-90	78.92	3.49	.395	4.42
Tahiti (Urban)	67	70-89	79.03	4.18	.510	5.29
Madagascar (Rural)	50	75-97	83.62	5.43	.768	6.49
U. Volta (Rural)	50	74-95	84.18	4.83	.683	5.73
W. Samoa (Urban)	50	74-99	85.78	6.43	.910	7.50
Selangor (Rural)	74	78-95	86.85	4.57	.531	5.26
Timor (Rural)	50	81-103	87.54	4.55	.644	5.20
Rajamundry (Urban)	50	83-102	91.18	5.11	.723	5.60
Rayavaram (Rural)	50	83-102	91.34	4.81	.680	5.26
Calicut (Urban)	50	84-103	91.30	5.01	.708	5.48
Varanasi (Urban)	50	84-103	91.84	5.08	.718	5.53
Ceylon (Urban)	50	80-100	91.92	4.88	.689	5.30
Egypt (Urban)	50	80-102	93.38	5.53	.781	5.91
Thailand (Rural)	50	85-103	96.24	4.42	.625	4.59

FIGURE 2

Range Diagram Showing the Number of Nuclei Between the Cephalic Space and Nerve Ring of Wuchereria bancrofti from Different Geographic Regions. The Solid Lines Indicate the Range, the Cross-Bar Indicates the Mean and the Box Encloses one Standard Deviation on Either Side of the Mean. Other Statistical Information is in Table 3.

FIGURE 2

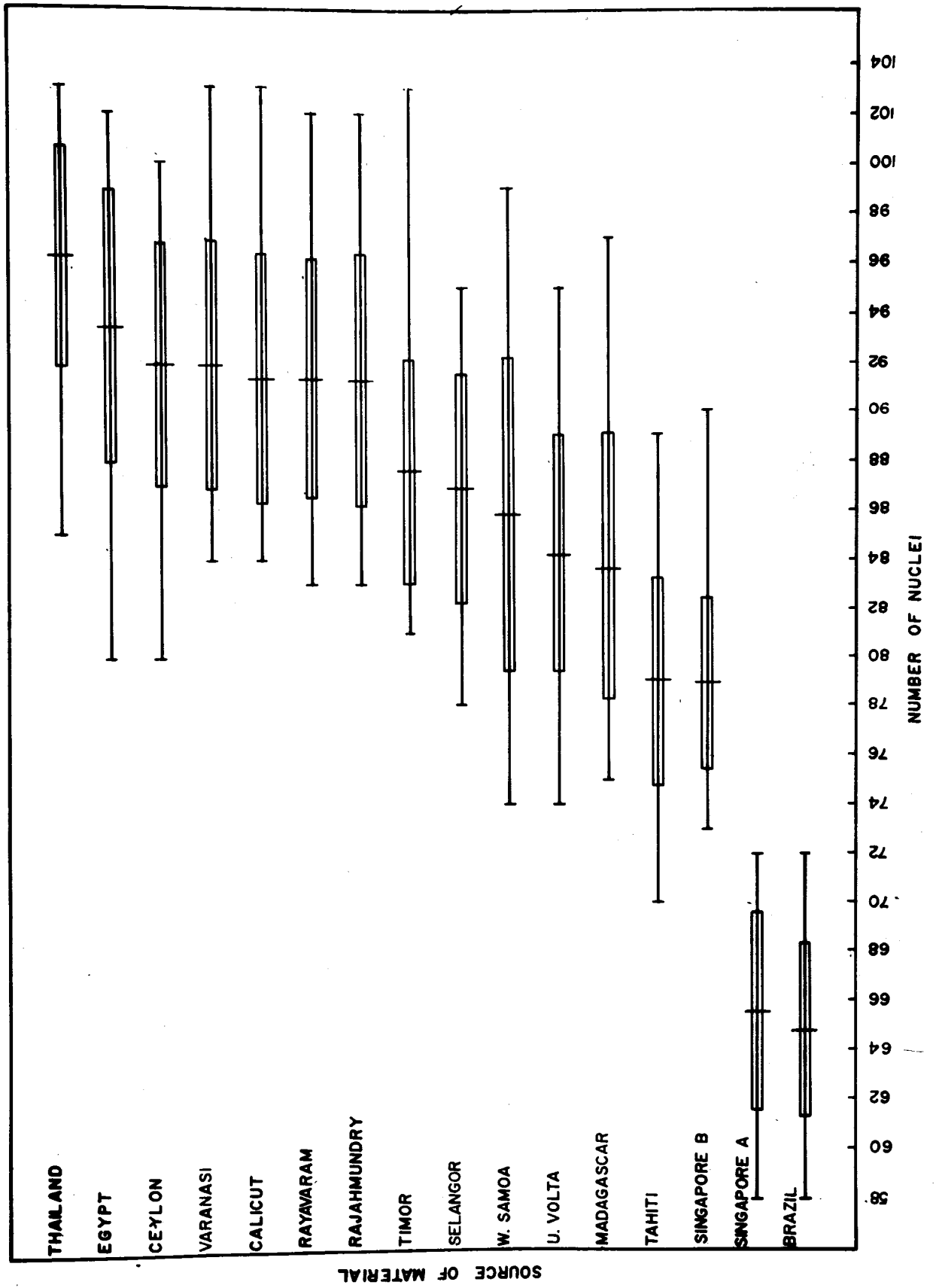
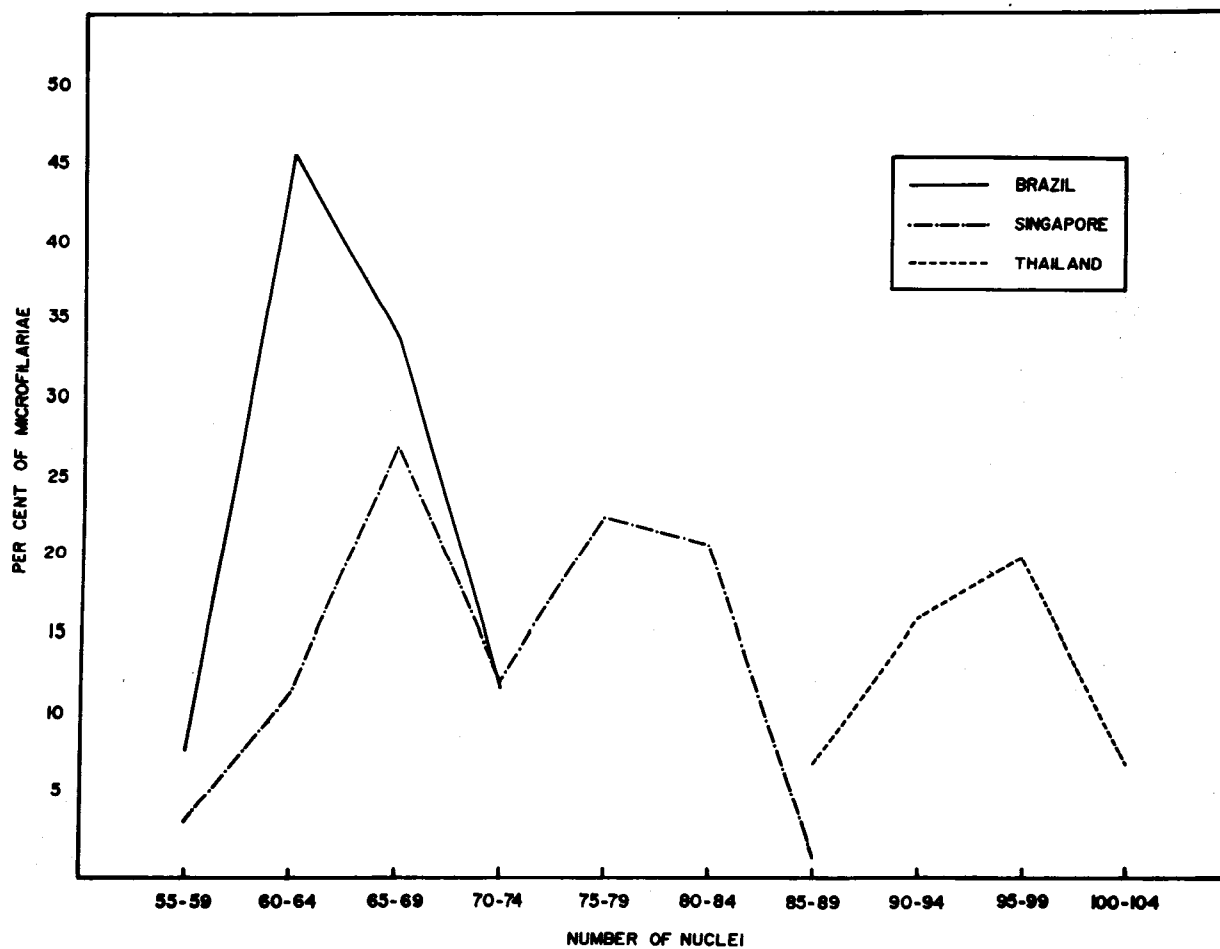


FIGURE 3



Frequency Polygon of CS-NR Nuclear Numbers of Microfilariae Collected in Brazil, Singapore and Thailand.

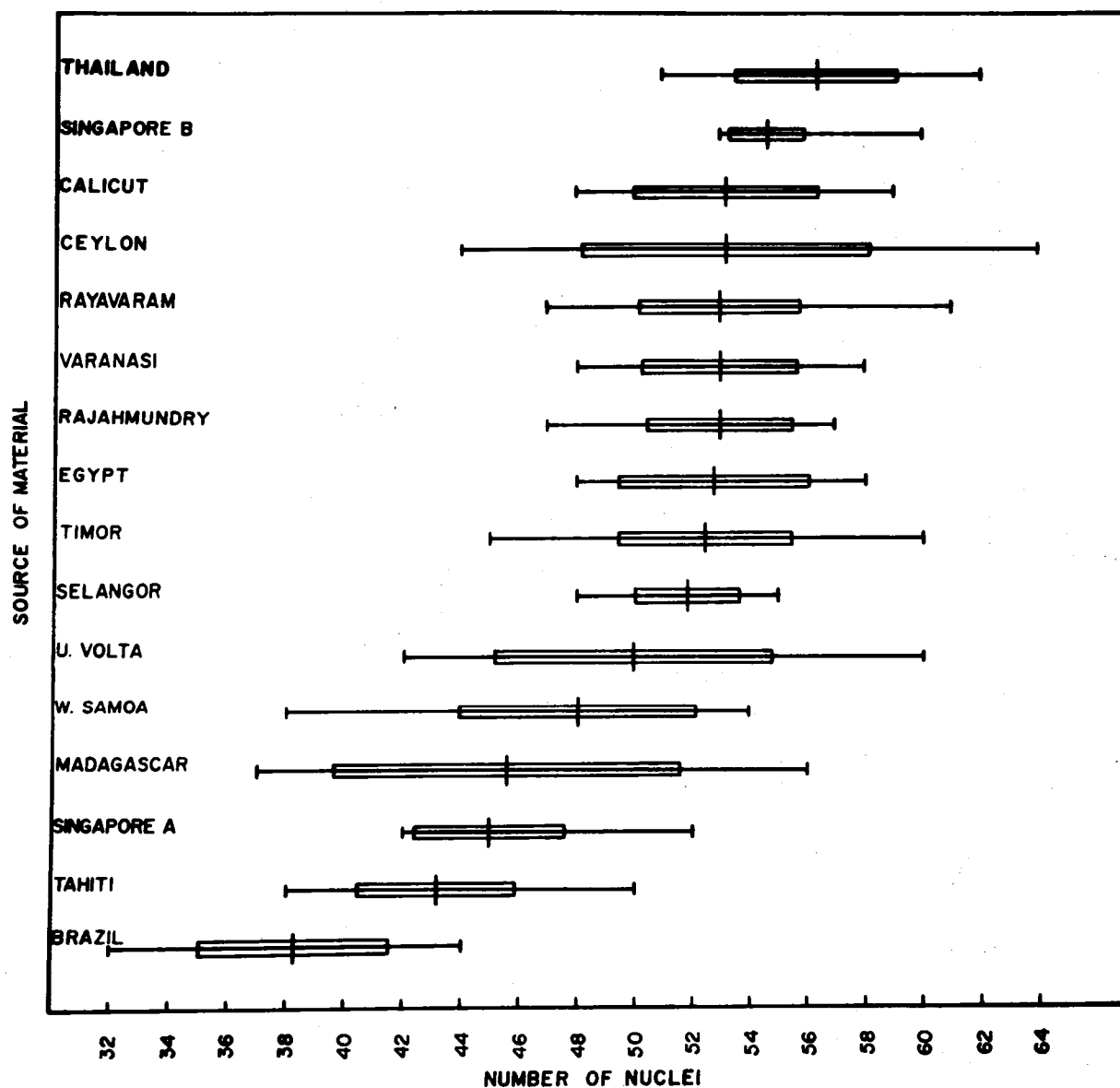
TABLE 4

Range, Mean, Standard Deviation, Standard Error  
and Coefficient of Variation in the Number of  
Nuclei Between the Nerve Ring and Excretory Pore  
of 33-50 Microfilariae of Wuchereria bancrofti  
from Different Geographic Regions.

Origin of material		Number	Range	Mean	S.D.	S.E.	V.
Brazil	(Urban)	39	32-44	38.26	3.23	.516	8.42
Tahiti	(Urban)	50	38-50	43.24	2.73	.385	6.31
Singapore A.	(Urban)	50	42-52	45.00	2.59	.367	5.76
Madagascar	(Rural)	33	37-56	45.58	6.06	1.053	13.28
W. Samoa	(Urban)	43	38-54	48.14	4.11	.625	8.50
U. Volta	(Rural)	44	42-60	50.05	4.81	.725	9.60
Selangor	(Rural)	52	48-55	51.87	1.81	.250	3.28
Timor	(Rural)	50	45-60	52.54	3.00	.424	5.71
Egypt	(Urban)	50	48-58	52.78	3.28	.464	6.22
Rajahmundry	(Urban)	48	47-57	52.96	2.57	.370	4.84
Varanasi	(Urban)	50	48-58	53.04	2.67	.377	5.03
Rayavaram	(Rural)	50	47-61	53.04	2.79	.394	5.26
Ceylon	(Urban)	50	44-64	53.16	5.05	.715	9.51
Calicut	(Urban)	43	48-59	53.23	3.19	.486	5.99
Singapore B.	(Urban)	50	53-60	55.16	1.30	.183	2.35
Thailand	(Rural)	47	51-62	56.38	2.81	.409	4.98



FIGURE 4



Range Diagram Showing the Number of Nuclei Between the Nerve Ring and Excretory Pore of Wuchereria bancrofti from Different Geographic Regions. The Solid Lines Indicate the Range, the Cross-Bar Indicates the Mean and the Box Encloses one Standard Deviation on Either Side of the Mean. Other Statistical Information is in Table 4.

TABLE 5

Range, Mean, Standard Deviation, Standard Error and Coefficient of Variation in the Number of Nuclei Between the Cephalic Space and Excretory Pore of 33-52 Microfilariae of Wuchereria bancrofti from Different Geographic Regions.

Origin of material		Number	Range	Mean	S.D.	S.E.	V.
Brazil	(Urban)	39	91-114	103.13	6.57	1.05	6.37
Singapore A.	(Urban)	50	107-129	113.94	4.66	0.66	4.09
Tahiti	(Urban)	48	114-136	122.15	5.85	0.84	4.78
Madagascar	(Rural)	33	112-148	130.64	11.04	1.92	8.45
W. Samoa	(Urban)	43	114-152	133.51	9.95	1.52	7.45
U. Volta	(Rural)	39	116-151	133.72	9.03	1.44	6.75
Singapore B.	(Urban)	50	128-150	135.58	4.16	0.59	3.07
Selangor	(Rural)	52	132-147	139.98	4.62	0.64	3.30
Timor	(Rural)	50	129-161	140.08	6.23	0.88	4.44
Varanasi	(Urban)	50	132-161	144.08	6.94	0.98	4.81
Rajahmundry	(Urban)	48	130-159	144.23	6.65	0.96	4.61
Rayavaram	(Rural)	50	131-161	144.28	7.06	0.99	4.89
Calicut	(Urban)	43	132-161	144.53	7.52	1.15	5.20
Ceylon	(Urban)	49	128-165	145.49	9.61	1.37	6.60
Egypt	(Urban)	50	129-160	146.36	7.75	1.09	5.29
Thailand	(Rural)	47	138-164	152.87	6.51	0.95	4.25

FIGURE 5

Range Diagram Showing the Number of Nuclei Between the Cephalic Space and Excretory Pore of Wuchereria bancrofti from Different Geographic Regions. The Solid Lines Indicate the Range, the Cross-Bar Indicates the Mean and the Box Encloses one Standard Deviation on Either Side of the Mean. Other Statistical Information is in Table 5.

FIGURE 5

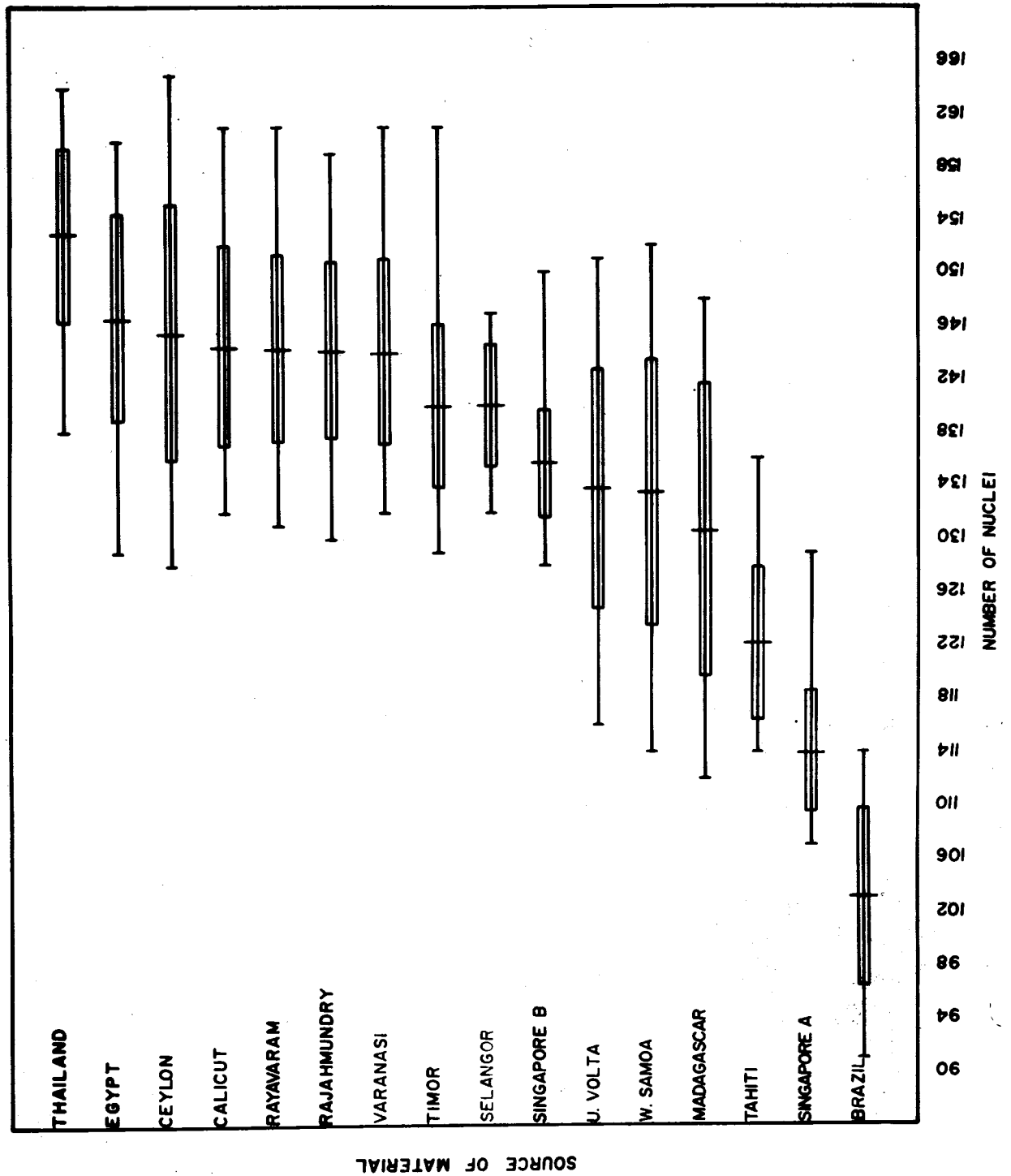


TABLE 6

Statistical Comparison of CS-NR Nuclear Number in Groups of Microfilariae from Different Geographic Regions Showing t-value and Probability of Their Being from the Same Universe. Cross Matchings not Found in This Table were Statistically Distinct ( $P = < 0.01$ )

Comparison	t-value	Probability
Singapore B. vs Tahiti	0.167	.84
Ceylon vs Varanasi	0.080	.92
Ceylon vs Rayavaram	0.701	.48
Ceylon vs Rajahmundry	0.740	.48
Ceylon vs Calicut	0.626	.55
Ceylon vs Egypt	1.400	.16
Timor vs W. Samoa	1.578	.11
Timor vs Selangor	0.823	.42
Varanasi vs Rayavaram	0.606	.55
Varanasi vs Rajahmundry	0.647	.55
Varanasi vs Calicut	0.535	.61
Varanasi vs Egypt	1.450	.13
Rayavaram vs Rajahmundry	0.060	.92
Rayavaram vs Calicut	0.061	.92
Rajahmundry vs Calicut	0.118	.92
Calicut vs Egypt	1.971	.40
Madagascar vs U. Volta	0.561	.55
Madagascar vs W. Samoa	1.813	.70
U. Volta vs W. Samoa	1.436	.16
W. Samoa vs Selangor	1.083	.32

TABLE 7

Statistical Comparison of NR-EP Nuclear Number in Groups of Microfilariae from Different Geographic Regions Showing t-value and Probability of Their Being from the Same Universe. Cross Matchings not Found in This Table were Statistically Distinct ( $P = < 0.01$ )

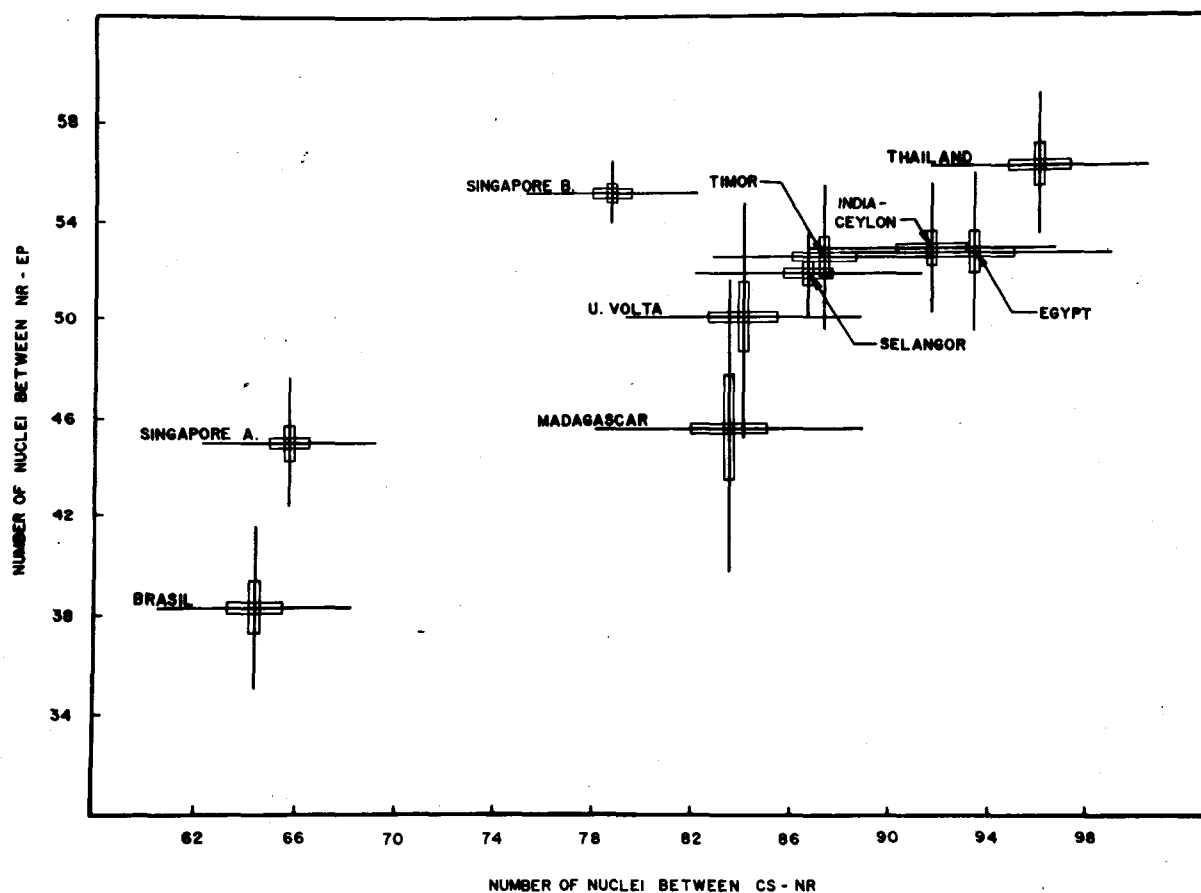
Comparison	t-value	Probability
Singapore A. vs Madagascar	0.438	.69
Ceylon vs Timor	0.745	.48
Ceylon vs Varanasi	0.148	.92
Ceylon vs Rayavaram	0.146	.92
Ceylon vs Rajahmundry	0.247	.84
Ceylon vs Calicut	0.081	.92
Ceylon vs Egypt	0.445	.69
Ceylon vs Selangor	1.735	.90
Timor vs Varanasi	0.879	.37
Timor vs Rayavaram	0.861	.37
Timor vs Calicut	1.076	.32
Timor vs Rajahmundry	0.739	.48
Timor vs Egypt	0.381	.69
Timor vs Selangor	1.380	.16
Varanasi vs Rayavaram	0.000	1.00
Varanasi vs Rajahmundry	0.154	.84
Varanasi vs Calicut	0.316	.76
Varanasi vs Egypt	0.434	.69
Rayavaram vs Rajahmundry	0.150	.84
Rayavaram vs Calicut	0.310	.76
Rayavaram vs Egypt	0.426	.69
Rajahmundry vs Calicut	0.453	.61
Rajahmundry vs Egypt	0.298	.76
Calicut vs Egypt	0.671	.48
Madagascar vs U. Volta	1.994	.40
Madagascar vs W. Samoa	0.483	.61

TABLE 8

Statistical Comparison of CS-EP Nuclear Number in Groups of Microfilariae from Different Geographic Regions Showing t-value and Probability of Their Being from the Same Universe. Cross Matchings not Found in This Table were Statistically Distinct ( $P \leq 0.01$ )

Comparison	t-value	Probability
Singapore B. vs U. Volta	1.293	.19
Singapore B. vs W. Samoa	1.339	.19
Ceylon vs Varanasi	0.362	.69
Ceylon vs Rayavaram	0.715	.48
Ceylon vs Rajahmundry	0.749	.48
Ceylon vs Calicut	0.525	.61
Ceylon vs Egypt	0.496	.61
Timor vs Selangor	0.091	.92
Varanasi vs Rayavaram	0.428	.69
Varanasi vs Rajahmundry	0.473	.61
Varanasi vs Calicut	0.230	.84
Varanasi vs Egypt	1.005	.32
Rayavaram vs Rajahmundry	0.036	1.00
Rayavaram vs Calicut	0.168	.84
Rayavaram vs Egypt	1.402	.16
Rajahmundry vs Calicut	0.205	.84
Rajahmundry vs Egypt	1.457	.13
Calicut vs Egypt	1.147	.27
Madagascar vs U. Volta	1.302	.19
Madagascar vs W. Samoa	1.189	.23
U. Volta vs W. Samoa	0.097	.92

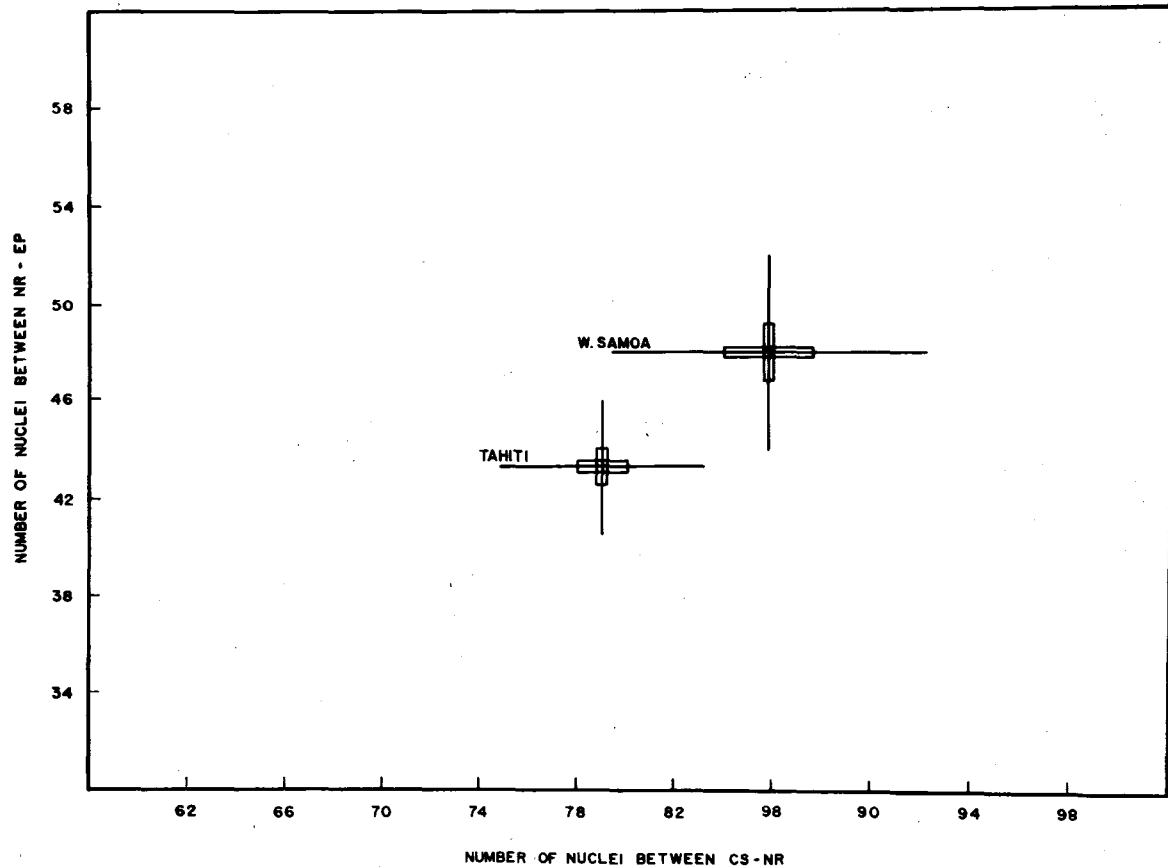
TEXT FIGURE VI



Bidirectional Graph Comparing the Number of Nuclei Between CS-NR (horizontal axis) and NR-EP (vertical axis) of "Periodic" Microfilariae from Different Geographic Regions. The Solid Lines Indicate one Standard Deviation, and the Box Enclôses Two Standard Errors on Either Side of the Mean.



TEXT FIGURE VII



Bidirectional Graph Comparing the Number of Nuclei Between CS-NR (horizontal axis) and NR-EP (vertical axis) of "Diurnally sub-periodic" Microfilariae from Different Geographic Regions. The Solid Lines Indicate one Standard Deviation, and the Box Encloses Two Standard Errors on Either Side of the Mean.

## DISCUSSION

The method of counting nuclei in Wuchereria bancrofti microfilariae, as used in this study, is simple and rapid, and avoids technical variations such as measuring errors. In essence, it is impossible to alter the number of nuclei, however crude the technique of slide preparation. The method does, however, require careful fixing and staining technique in order to demonstrate individual nuclei to best advantage. Fifty microfilariae of Wuchereria bancrofti from each of the fifteen geographic areas were examined for their nuclear numbers between CS-NR and NR-EP. This probably presents the largest series of samples of a single species thus far investigated in regard to eutely.

We feel that the almost exact coincidence of nuclear numbers in the Indian (Varanasi, Rajahmundry, Rayavaram and Calicut) and Ceylonese material (Tables 6, 7, 8; Figs. 2, 4, 5), despite the wide geographical separation between locales, best demonstrates the validity of the method in assessing relationships between strains from different areas. (The geographic distance between Varanasi and Rajahmundry is approximately one thousand kilometers, nearly the same as between England and Italy). The close numerical relationships between Indian-Ceylonese material and that from Egypt probably indicates a common ancestry for the strains found in both regions, as also is the case in strains from Selangor and Timor.

Two biologically distinct forms of Wuchereria bancrofti have been recognized for many years. The periodic form, in which the microfilariae show nocturnal periodicity in the peripheral blood, has almost worldwide distribution in tropic and sub-tropic zones; the diurnally sub-periodic

form is restricted to the Polynesian Islands (Faust and Russell, 1964; Text fig. 1). Despite differences in microfilarial periodicity and vector, adults of the two forms of Wuchereria bancrofti are roughly similar overall in their morphology. Tabulations of differences, mainly of size and shape were published by Fain (1951) and Buckley (1952). Although attempts have been made to assign taxonomic status to the diurnally sub-periodic form (Wuchereria bancrofti var. pacifica Manson-Bahr and Muggleton, 1952), Buckley, l.c. and Buckley and Singh (1965) were unwilling to establish this form as a separate species until differences were confirmed in an adequate range of specimens.

The present study gives strong morphological evidence based on statistical arguments that Wuchereria bancrofti can be regarded in a broad sense as a species complex or group of sibling species (Mayr, 1964, 1966). We feel that morphological differences between microfilariae combined with such strong biological differences as periodicity should be sufficient to support establishment of the Pacific form as a distinct sub-species, Wuchereria bancrofti pacifica (Manson-Bahr and Muggleton, l.c.) or possibly even full species status. Until more material from the Pacific region can be studied to determine the range of variability, however, no formal taxonomic proposals are contemplated.

Text figure 6. shows that isolates from Brazil and Singapore A. are quite distinct both from each other and from other strains; the remainder of the nocturnally periodic material appears to form a cline of intergrading forms. The smallest number of nuclei in both range and mean between CS-NR, NR-EP and CS-EP were found in material from Brazil (Tables 3-5). There were significant differences between this material and all of the other isolates in CS-NR, NR-EP and CS-EP nuclear numbers. It is possible that these

differences would justify erection of a further new sub-species, but formal erection of a taxon must await confirmation of findings on more isolates.

The Singapore A. form differs from all other isolates in both CS-NR and CS-EP nuclear numbers (Text Figs. 2 and 5), but is similar to Madagascar in NR-EP number (Text fig. 4, Table 7). Two infra-specific forms of Wuchereria bancrofti apparently occur in Singapore, possibly one of Indian and the other of Chinese origin. Unfortunately, we were not able to obtain material from China for comparison. Until more samples are studied from this area, the taxonomic status of this form should be left open.

The similarity of material from Madagascar to that from Upper Volta and in one respect to Singapore A. (Tables 6-8; Text fig. 6) casts doubt on the status of Wuchereria vauceli (Galliard, 1959) as an independent species. We feel that it should be suppressed as a synonym of Wuchereria bancrofti (Cobbold, 1877) Seurat, 1921, wherein it would form part of the cline which appears to comprise the nominal sub-species.

The most probable explanation for differences in nuclear numbers between microfilariae of Wuchereria bancrofti from different geographic regions lies in the adaptation of the parasite to a new invertebrate host in order to survive in a new or changing environment. Continuing selection by vectors may result in morphological differences becoming apparent and stable.

Considerable adaptive pressure is placed on filarial worms taken from one area to another (i.e. from one vector to another), hence selection and evolution of different morphological or physiological strains would possibly be more rapid than in the case of nematodes with a direct life cycle. If adaptation is necessary, and if evolution is a natural consequence of selection on the basis of adaptation, it follows that the more often a filaria

is transplanted into another ecological situation, the more variation should be possible and the more rapidly it could come about.

Man and Wuchereria bancrofti appear to be relatively well adapted to each other. This adaptation has taken place over a long period of years following the initial acquisition of infection, presumably with an animal strain. The parasite has radiated with man from a central focus or foci and lost its adaptability to animals, and as man has geographically isolated himself in different ecological niches, he has also forced isolation and adaptation upon his parasite. Most of this adaptation must, we feel, have taken place by vector selecting mechanisms. A vast variety of mosquitoes may act as vectors: nocturnally feeding mosquitoes of the Culex pipiens group, Anopheles and Mansonia in various parts of the world, and diurnal Aedes spp. in the Pacific islands (de Rooek, 1957). This wide range of mosquito hosts confirms that adaptation has taken place (Wharton, 1963). Re-adaptation to vertebrate hosts other than man has, on the other hand, not been observed. Apparently, as long as a mosquito population has sufficient contact with man, the parasite is as yet sufficiently flexible in its requirements to survive. Yet, the fact that Wuchereria bancrofti is not present in all tropical environments means that there are conditions which are frequently difficult to meet (Wharton, l.c.).

Presumably, the ability of Wuchereria bancrofti to develop in a formerly suitable mosquito host may be lost if the parasite becomes adapted to a new vector. This was recently illustrated by two strains of Wuchereria bancrofti in Malaya, one urban and transmitted by Culex pipiens fatigans, the other rural and transmitted by a species close to Anopheles letifer (Wharton, 1960, 1963). The latter strain has lost its ability to use Culex pipiens fatigans as an intermediate host.

Rosen (1955) found that a Tahitian strain of Culex pipiens fatigans was a good host for a strain of Wuchereria bancrofti from Martinique (West Indies), but was inhospitable to the local sub-periodic strain. Backhouse and Woodhill (1956) showed that Culex p. fatigans from Australia was a good host for sub-periodic Wuchereria bancrofti in New Caledonia, where the vector is yet another mosquito, Aedes vigilax. This mosquito has recently been introduced into Fiji, but Burnett (1960) has shown that Fijian strains of Wuchereria bancrofti do not develop readily in this new arrival. Whereas 94-95 per cent of Aedes vigilax from Sydney or from New Caledonia become infected after feeding on sub-periodic strains of Wuchereria bancrofti of New Caledonian origin, only 6 per cent of the Aedes vigilax in Fiji became infected after feeding on a local sub-periodic strain. Burnett (l.c.) remarked that "it is very probable that difference is not in the strain of mosquito but in the strain of parasite, although both are sub-periodic".

The ability of a given mosquito species to act as a good vector is under genetic control as shown by Macdonald (1962), who was able selectively to breed two different strains of Aedes aegypti, one almost completely susceptible to infection with sub-periodic Brugia malayi, the other almost totally refractory. A recessive gene present on the sex chromosomes was found to control the vector's susceptibility. The same gene has been observed to exert a similar control over susceptibility to periodic and sub-periodic Wuchereria bancrofti (Macdonald and Wharton, 1963). Many variations in the vector potentialities of the same mosquito in different parts of its range can thus be explained by strain compatibilities or incompatibilities in either parasite or vector.

In the Pacific islands, selection of a compatible strain of parasite imposes a double adaptive challenge. During the Polynesian migrations, when

Wuchereria bancrofti probably was introduced into the Pacific islands, microfilariae were presumably nocturnally periodic. The mosquito fauna was limited, however, and the Aedes scutellaris complex, diurnal in its feeding habits, constituted the dominant part. We agree that the evolution of sub-periodic strains of Wuchereria bancrofti in the Pacific islands would be the result of natural selection as postulated by Iyenger (1965). Although information on the longevity of filarial infection in man is limited, it has been shown that microfilariae may persist in an individual without reinfection for about five years (Jackowski et al., 1951). This would provide a reasonably long period during which contact might be established between the microfilariae and potential vectors in new localities.

One possible point of utility arising from the present study is to afford a method for the investigation of the anthropological and historical affinities of the vertebrate host. We feel that if the morphological types of Wuchereria bancrofti microfilariae are identical in two geographical isolates, it would tend to show a relationship: racial, commercial, or historic between the peoples of those two regions; if different, it would help to point out situations that anthropologists could then investigate using other criteria. The value of using Wuchereria bancrofti in preference to other helminths is that it is thus far found only in man.

Despite a large amount of literature on human racial origins, racial relationships and migrations, there is little direct evidence on which to base conclusions. It is not yet known whether Homo sapiens evolved from a single prehuman species or began as hybrid between two or more. Many anthropologists feel that the various racial divisions commonly used to classify modern human varieties are not descended from different sub-human ancestors (Taylor, 1927).

The concept previously held by anthropologists was one of clearly differentiated Negro, Caucasoid, and Mongoloid original stocks, and that the many intermediate types now linking the extremes of each stock were the result of crossing. The earliest finds of true humans do not substantiate this idea in any way (Taylor, l.c.). Early man seems to have spread over the old world rapidly until he occupied all but the circumpolar and most inhospitable desert regions. The extinction of other human types during the late Pleistocene as Homo sapiens occupied the earth may have been accomplished more by disease than by mixture (Taylor, l.c.).

Indigenous helminth fauna indices were used by Von Thring (1902; in Hegner et al., 1938) to determine the origin of different South American Vertebrates, especially mammals. He discussed the possibility of their being indigenous to South America or alternatively, arising north of the Isthmus of Panama and then spreading southward. He emphasized the point that two host species, parasitized by the same or by nearly related species of parasites, are themselves of common descent. Darling (1925) based his speculations on the origin and spread of the human races by the use of information on hookworm distribution. Similar uses of parasite data have been made by other workers (Zschokke, 1904; Kellogg, 1905; Johnston, 1914; Metcalf, 1929; all in Hegner, l.c.).

The complete overlap in two directions of one Ceylonese and four Indian strains as well as the similarity of material from Selangor to that from Timor (Tables 6-8; Text fig. 6) probably is an indication of the close anthropological and historical relationship between the peoples in the respective areas. According to Williams Hunt (1952) the aborigines (Pagan hill peoples, "Negrito" or "Sakai" ) of Selangor belong to three distinct



stocks of Siamese, Malay and Negrito. Six skulls from various Malayan caves and rock-shelters were studied by Duckworth (1934; in Coon, 1963) and found to follow a single pattern, that of the "Melanesians". In Timor the bulk of the population is Papuan (Melanesian or Australasian aborigines), intermingled in various proportions with Malayo-Indonesians (Gilbert, 1949). Our findings might thus support the hypothesis that early migrants from South-East Asia entered Australia by way of Timor using the Timor-Sahul shelf route when the great shelves were above sea level (Coon, l.c.).

Nothing is known of the origin of Wuchereria bancrofti nor are there any indications of animals other than man acting as a host; however, too few surveys of animals for this parasite have been made to be of absolute assurance. The finding of Brugia malayi as a zoonosis imposes considerable caution in this regard (Nelson, 1965). In East Africa, where periodic Wuchereria bancrofti is common in man, Buckley et al. (1958) could not find this parasite in dogs, cats, and genet cats: Nelson et al. (1962) also examined a wide range of animals in this area with negative results. In the areas of sub-periodic Wuchereria bancrofti Symes (1960) in Fiji, and Beye et al. (1952) in Tahiti examined a number of animals and birds, also with negative results. Wharton (1960) stated: "There might be an animal reservoir in areas such as Annam Range, where Canet (1952) recorded non-periodic bancrofti among Malay-Polynesian people."

Attempts to transmit Wuchereria bancrofti to animals have been too few (Laing et al., 1960; Ramachandran et al., 1966) thus far to assess accurately the reasons for their failure. From a theoretical point of view, these could be several: (1) Complete physiological incompatibility

between the parasite and the host species selected (2) Growth requirements may have been met, but worms were not recovered for technical reasons (3) Worms may have matured but did not mate (or only worms of one sex matured) or, if mating occurred, females did not produce microfilariae (4) if microfilariae were produced, they were trapped in lymph nodes or blood vessels of the viscera.

It is not possible from our present state of knowledge to conjecture on the type of host animal best suited for transmission experiments. Following the present study, it may however, be possible to postulate strains of worms better suited for such attempts than others. We feel that lower nuclear numbers probably indicate more primitive and possibly less strongly human-adapted strains. As sub-periodic strains have probably derived from periodic strains, the latter would be the less specialized. For this reason, it appears to us that transmission experiments should be instituted using the Brazilian strain of parasite in a variety of potential hosts.

A final consideration arising from the present study would be to extend the present work to other filarial worms: the relationships of old and new world Onchocerca (Strong, 1937 in Nelson, 1965; De Leon and Duke, 1966; Woodruff et al., 1966); the relationships between Loa loa and Loa papionis (Treadgold, 1920 in Nelson, l.c.; Duke and Wijers, 1958) and of Mansonella ozzardi in Brazil and elsewhere (Buckley, 1934; Biagi, 1956, 1957; Biagi et al., 1958; Garnham and Walliker, 1965).

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