REVIEW

Early History of Laboratory Breeding of Aedes aegypti (Diptera: Culicidae) Focusing on the Origins and Use of Selected Strains

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ABSTRACT

The yellow fever mosquito, Aedes aegypti (L.) (Diptera: Culicidae), is well recognized for its extensive adaptation to diverse ecological conditions and for genetic variation. Recognizing the importance of strain variation of this mosquito, researchers have established a large number of laboratory strains. Some of the popular strains have been used for research for years in many laboratories around the world. However, the exact origins of many of these strains are unknown. In this review, publications and archival records were examined to report the early laboratory mosquito rearing practices around the world and to identify the origins of selected strains. The records showed that inter-laboratory sharing of strains was already underway in the early part of the 20th century because of the ease of breeding Ae. aegypti and of sending eggs by mail. It also was found that the four strains established in major U.S. institutions by the mid-1930s, including the “ROCK” (short for Rockefeller) strain, had been derived from Cuba, Nigeria, Philippines, or Puerto Rico, all known for a long history of transmission of yellow fever virus or dengue virus rather than from North America. The strains used for research in Europe were primarily derived from West Africa, but strains of Asian, Caribbean, and South American origins also were used for comparative experiments among geographic strains. Neglected issues related to strain designation and original source identification in scientific publications were found and their relevance to current research is discussed.

KEY WORDS Aedes aegypti, laboratory strain, source identification, historical review

“What is the importance of laboratory colonies of Aedes aegypti to science?” “A review of Tropical Diseases Bulletin between 1960 and 1964 indicates that between one-third and one-half of all references on mosquitoes deal with Aedes aegypti. The book on mosquito physiology by A.N. Clements contains 800 references and 400 of these are on Aedes aegypti. When one recalls there are 2,400 species of mosquitoes, this does seem disproportionate.” “A. aegypti has become as standard for the experimentalists as the Wistar white rats or Drosophila melanogaster.” “Therefore, it is of immeasurable usefulness to the advancement of our knowledge of insects and of biological knowledge in general” (Craig 1965a). The late George Craig used these words to defend research using laboratory strains of Aedes aegypti (L.) (Diptera: Culicidae) in response to a proposal to severely restrict use of this mosquito during the eradication campaign waged in the Western Hemisphere.

Ae. aegypti continues to have significant impact and importance as a research tool and also for its importance as a vector of dengue virus (family Flaviviridae, genus Flavivirus, DENV), yellow fever virus (family Flaviviridae, genus Flavivirus, YFV), and chikungunya virus (family Togaviridae, genus Alphavirus, CHIKV).

The species has enormous capacity to adapt to diverse ecological conditions. Its many biological and phenotypic variations exhibited by this species clearly reflect great genetic plasticity (Coker 1967, Tabachnick and Powell 1979), including emergence of insecticide-resistant subpopulations shortly after the end of World War II (WWII). Ae. aegypti is the primary urban vector of DENV that is the only virus (among nearly 550 arboviruses) transmitted between only one vector (Ae. aegypti) and humans as long as the size of susceptible human population and other conditions remain favorable (Kuno and Chang 2005). The physiological basis of this specific vector–human relationship is unique (Harrington et al. 2001). The diversity of the species throughout the world is also critically important for designing better strategies for controlling this mosquito (Service 1992).

In addition to the importance of Ae. aegypti, the large number of scientific publications on the species is the result of the ease of laboratory breeding and of convenience of colony sharing among scientists through shipment of eggs by mail.

The accuracy of the identities of different laboratory strains (hereafter called “strain” instead of colony) is not critically important for some studies so long as the identity of the species used is correct. However, for studies that seek a comparative analysis, such as

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investigations of differences of particular biological or genetic traits among subpopulations of the species, precise identity of the strain is critical.

The genome sequencing and other characterization techniques have become an integral component of Ae. aegypti strain characterization. This is illustrated in online information resource, i.e., VectorBase managed by the National Center for Biotechnology Information of the National Institutes of Health (NIH) (http://vectorbase.org/Help/EST_Libraries/Aedes_aegypti). In such a database, too, accurate identity of each strain used to deposit data has become important.

It has been recognized for many years that exact origins of some strains popularly used in many laboratories were unknown. For example, the origin of the ‘ROCK’ (short for Rockefeller) strain was reported to be unknown even before 1950 (Downs and Baker 1949). Although general lack of interest in history among many scientists may be one of the reasons, a preliminary study conducted for this review uncovered multiple, troubling practices in documenting source information for laboratory strains up to the present time. These problems are discussed in the last section of this review and were the major sources of limitations for a retrospective identification of the origins of both old and recently derived strains.

Objectives

The first objective of this review is to identify the origins of selected Ae. aegypti strains through published documents and other archival records. A survey of laboratory breeding practices for Ae. aegypti in the nineteenth and early twentieth centuries is presented to review the historical background that influenced strain establishment and to highlight the major research activities that used the strains. This review seeks to fill a gap in nearly all historical reviews and books on Ae. aegypti. Information has been unavailable on the origin, characteristics, and significance of specific strains used. If a question of authenticity of a particular strain arises as part of a reassessment of past data, a historical review such as this provides useful information. Finally, a variety of the problems related to strain designation and recording practices were revealed in this review that remain a concern to the present day.

Retrieval of Laboratory Strain Source Information and Breeding Records

Scope of Literature Search and Limitations. The scope of this historical review of Ae. aegypti strains established before 1945 was limited to eight strains established in the United States and United Kingdom, whereas a general survey of laboratory breeding covered all regions of the world. Only three U.S. records before 1945 were included because the number of records in the country after 1941 is too many to be covered adequately. Although the publications before 1945 are the main focus of this review, a small number of publications thereafter are included for the discussion of selected topics either to enrich discussion, to clarify troubling issues, or to cite specific examples. The limitations of retrieving original strain sources due to a variety of problems are listed in the last section of this review.

Synonyms. The following synonyms of Ae. aegypti were used for information retrieval from the biomedical literature: Aedes (Stegomyia) aegypti Linnaeus; Aedes (Stegomyia) fasciata F.; Stegomyia fasciata F.; Aedes (Stegomyia) argentatus Poirier; Stegomyia calopus Meigen; and Aedes calopus Meigen.

Definition of “Strain.” An established laboratory strain that can be maintained for a long period typically meets the following minimal requirements: it has a record of laboratory maintenance for more than several generations or for more than a year without replenishment. A strain is readily amenable for mass production; the life stage is easily synchronized; and, if requested, a sufficient number of eggs can be readily shipped to other institutions. In the early part of the history of mosquito breeding, a small number of laboratory breeding records met the requirements. Otherwise, the status of laboratory establishment probably fell in the category of temporary breeding for the duration of study rather than long-term establishment. However, a clear distinction between temporary breeding and true strain establishment is difficult to access in too many publications. This is because of the absence or ambiguity of records, which reflected the prevalent styles of writing and standards in scientific writing in that period. The word “strain” is used in all reports of laboratory breeding whether or not a strain meets today’s standard.

Strain Designations (Names). For many commonly used strains today, the strain designations in the lists prepared previously (Craig et al. 1961, University of Notre Dame 1961, World Health Organization 1968) are used directly within quotation marks. Similarly, for other strains not in these lists, the adopted strain designations found in publications are used with a quotation mark. If no adopted strain name was found in the literature, it is presented simply as a strain together with the name of the originating institution.

Technical Advancement and Events that Influenced the Use of Ae. aegypti Strains in Research

Improvement in Rearing Techniques. From the latter part of the nineteenth century to the early twentieth century, the physical size of laboratory-bred mosquitoes was typically small but adequate for systematics and bionomics studies. Lack of knowledge about the mechanisms of egg hatching, optimal larval nutrition and bloodmeal for adults, and a reliable method for preserving viable eggs for a long periods rendered methods were inadequate for research requiring a large number of mosquitoes at a particular life stage. By the beginning of the second decade of the twentieth century, breeding procedures improved considerably with respect to more consistent egg hatching through discovery of microbial growth that reduced oxygen concentration as essential stimulants
of hatching, better sources of bloodmeal, and technical advances, such as improved design of mosquito cages, development of pupal separators, and other improved facility design. By 1937, a mass rearing procedure was deployed in the U.S. Public Health Service for a large-scale drug screening (Johnson 1937).

**Historical Events.** Between 1870 and 1950, several events contributed to increased interest in and use of laboratory-bred *Ae. aegypti*. The first events were of course the chain of major discoveries resulting in the recognition of vector-borne transmission of human and animal pathogens, including confirmation of mosquito-borne transmission of YFV by the Walter Reed Commission and identification of *Ae. aegypti* as vector of dengue by Thomas L. Bancroft. In this early period, biological traits and replication of some pathogens not associated with *Ae. aegypti* in nature were nonetheless studied with this species, because it was then the only readily available, laboratory-bred mosquito species.

In parallel with the progress in pathogen–vector relationship studies, laboratory strains of the species were frequently used for bionomics, physiological, and genetic studies. The rising interest in studying mosquitoes was also reflected in the opening of the schools dedicated to tropical medicine in Liverpool and London in 1899.

As consequence of the outbreak of WWII, the U.S. Army, to protect its troops in the tropics, invited 63 medical schools to participate in courses in tropical medicine and field experience (Simmons 1947). Also, both the U.S. Army and Navy increased the number of personnel involved in medical entomological and tropical disease research and control (Armed Forces Pest Management Board 2008). The wartime mobilization for vector research and control programs also involved internal reorganization within the military, Department of Agriculture, and Public Health Service.

The huge increase in the number of scientists in research and academic institutions as well as in military organization was reflected in a nearly 46% increase in membership of the American Society of Tropical Medicine and Hygiene between 1942 and 43 (American Society of Tropical Medicine 1944). Screening and efficacy evaluation of a large number of new insecticides (including DDT) and repellents, as well as for research on insecticide application techniques (such as ultra low volume technique) were conducted with a local *Ae. aegypti* strain in the Orlando facility of the U.S. Department of Agriculture (USDA).

Until around mid-1930s, most U.S. laboratories maintained only one *Ae. aegypti* strain. However, British and French researchers, recognizing the importance of strain differences, had multiple strains. In the United States, the expansion of research on tropical diseases and vectors during WWII was the major cause of a sharp increase in strain sharing among laboratories, resulting in many institutions maintaining multiple strains.

**Origins of Selected Strains Established Before 1945**

“ROCK” Strain. Wilhelm H. Hoffmann was a German Navy physician with a long experience in epidemiological investigation of YFV in Africa and Brazil. He had been invited by Juan Guiteras to Cuba as director of the Carlos Finlay Institute in Havana in 1916 (Hoffmann 1942, Ramos Báez 1950, Hinz-Wessels 2008). In 1926, he shipped *Ae. aegypti* eggs from the strain maintained at the institute to the Department of Tropical Medicine at Harvard University at the request of Andrew W. Sellards (Gay and Sellards 1927). Around 1935, eggs from this strain were shipped from Harvard University to the Department of Animal and Plant Pathology of the Rockefeller Institute of Medical Research at Princeton, NJ (RFIMR-Princeton) (Merrill and Ten Broeck 1935), where the strain was maintained by William Trager. Eggs were shipped later from RFIMR-Princeton to the RFIMR Laboratory in New York (RFIMR-NY), where the strain was maintained by Loring Whitman. In 1936, the strain was shipped by Trager to the Rutgers University (Granett and Powers 1937, Sutherland 1964: Fig. 1). In 1938, the eggs were shipped from RFIMR-NY to Dale W. Jenkins of the U.S. Army at Fort Detrick, MD. In 1959, the eggs from this strain were shipped from Jenkins at Fort Detrick to George B. Craig of the University of Notre Dame (Dunn and Craig 1968: Fig. 1). Because no strain designation was used in any of the first three laboratories above, including the Finlay Institute in Havana, apparently the strain name “ROCK” was coined in one of other receiving institutions. However, it is probable that the “ROCK” designation originated at Ft. Detrick because by 1959 the code name at Ft. Detrick of this strain was “R” (Rockefeller) (Hay 1999).

The history of “ROCK” strain sharing depicted in Fig. 1 illustrates only the known transactions recorded in publications that were identified in this literature review for the covered period. It may not represent all of the transactions that may have occurred.

The first documented strain of *Ae. aegypti* was established in Havana, Cuba, by Carlos J. Finlay in 1881 (Finlay 1881). This strain was used by the Walter Reed Commission during the yellow fever studies in Havana in 1900–1901 (Fig. 1). After Finlay’s death in 1915, the Finlay Institute was established by the government of Cuba in his honor. Although no record was found confirming that the strain maintained at the Finlay Institute after Finlay’s death was Finlay’s strain, this possibility is very strong. If so, the strain sent by Hoffmann to Harvard in 1926 was the same strain Finlay had established as early as 1881. Assuming that the “ROCK” strain currently used is authentic, then this strain would represent the longest, uninterrupted lineage of an *Ae. aegypti* strain in the world, spanning nearly 130 yr.

**The Earliest Strain at the U.S. Army Medical School.** In 1925, Joseph F. Siler established a strain for transmission experiments with DENV in Manila (Siler et al. 1925). That year, the strain was shared with the Army Medical School, Washington, DC, and was used...
for experiments (Kelser 1933). This strain was used throughout repeated reorganizations of the medical department of the U.S. Army and renaming of the School the Walter Reed Army Institute of Research. Under the direction of James S. Simmons, the strain was used for confirmation of vector-borne transmission when Albert B. Sabin isolated DENV in 1944–1945 during WWII (Sabin 1952).

In the wake of the outbreak of WWII, the U.S. Army established a research facility at Camp Detrick, MD, in 1943. After the facility was established but before its renaming as Fort Detrick in 1956, a strain of *Ae. aegypti* designated “CD” (short for Camp Detrick) was used there. Its origin remains unknown. “CD” was not identical to the “ROCK” strain, because both strains were used distinctly at Ft. Detrick in the 1950s (Hay 1999). Probably, the “CD” strain was the same as the strain used by the Army Medical School that was obtained through intramural sharing of the strain. This speculation is plausible because it was the quickest way to commence research using this mosquito at the new facility. Accordingly, the “CD” and “FD” strains from the Ft. Detrick facility (Craig and Gillham 1959) are also identical to the original strain obtained from Manila and maintained at the Army Medical School. However, the “Fort Detrick” strain that is found in the literature was different because it was obtained by the Ft. Detrick facility from the Bureau of Entomology and Plant Quarantine of the USDA well before 1956 (Casida 1955, Craig et al. 1961). Accordingly, it was probably derived from what is currently known as the “Orlando” strain that had been originated in either 1939 or 1942.

The Original Strain at the Johns Hopkins University. In 1934, Lloyd E. Rozeboom of the School of Hygiene and Public Health at the Johns Hopkins University (JHU) received eggs from William A. Hoffman then of the Department of Tropical Medicine at the University of Puerto Rico, Río Piedras, Puerto Rico (Rozeboom 1935). The strain was maintained by Paul A. Woke until his departure for military service during WWII. It is probable that this strain was sent to Harvard University. The strain received at Harvard from the JHU was recorded to have its establishment at the JHU well before 1944 (Spielman 1964). The fate of this strain thereafter at the JHU is unknown, because it was not found among many strains used at the JHU after WWII. The fate at the Harvard University is also unknown because of a transient financial difficulty of the Department of Tropical Medicine to support its programs and the untimely death of A. W. Sellards in 1942. In fact, the strain used after 1965 was obtained from the Grand Bahama Island (Spielman et al. 1967).

The Original “NIH” Strain. Identification of the exact origin of the so-called “NIH” strain (University of Notre Dame 1961) is difficult, because of the long and complicated history of repeated internal reorganizations in the U.S. Public Health Service (USPHS), renaming of institutions, and the establishment of multiple laboratories all using a strain of *Ae. aegypti* for research within the NIH. The earliest record of breeding *Ae. aegypti* by the staff of USPHS was a short-term
experiment conducted in Mexico for yellow fever investigation by the members of Marine Hospital Service in 1904–1905 (Rosenau and Goldberger 1906). The exact date of the establishment of a strain in a USPHS laboratory on U.S. soil has been ambiguous and ranged from as early as in 1921 to as late as in 1938, all based on recollection (VandeHey 1964, Leahy and Craig 1967) but not on actual documents. The first record documenting establishment occurred at the Rocky Mountain Laboratory (RML) in Montana, where in 1930 Cornelius B. Philip received eggs from his former colleague of the Rockefeller West Africa Commission, Henry Beewuks, in Lagos, Nigeria (Philip 1932). This date also agrees very well with the presumed date recorded at the University of Notre Dame when the strain was received from Woke of the NIH in 1938 (Leahy and Craig 1967).

The origin of the strain available by 1937 at the Laboratory of Tropical Diseases (LTD) (Johnson 1937) is unknown. However, the strongest possibility is intramural sharing of the strain available at the RML since 1930, because within a year after the LTD was established, a research paper about mass rearing of this mosquito was published from LTD in 1937. Still, it is not entirely certain if this strain was identical to the strain shipped from P. A. Woke, who joined LTD in 1954, to the University of Notre Dame. Similarly, the source of a strain used by Helen L. Trembley first in the Division of Physiology in 1944 (Trembley 1944) and later at the LTD (Trembley 1952) remains unknown. Nonetheless, among multiple possibilities, it is highly likely that the strain used by Trembley was the source of the “NIH” strain because Woke was assigned to the LTD and became responsible for the breeding of mosquitoes in 1954.

A Strain at USDA. Leland O. Howard worked with an *Ae. aegypti* strain for his systematics, bionomics and larval control (with kerosene) studies sometime between 1892 and 1912 at the Bureau of Entomology, Washington, DC.

The Bureau of Entomology and Plant Quarantine in Orlando, FL, which had existed since 1930, was more engaged in citrus pest than in mosquito research. The earliest date of the establishment of a strain derived from locally obtained mosquitoes by the USDA probably occurred after a new laboratory was established for medical entomological research in nearby New Smyrna Beach in 1938 or in 1942 when the laboratory in Orlando was reorganized exclusively for medical entomological research. Thus, both the original dates of 1939 and 1942 are possibilities (Travis et al. 1946, Schreck et al. 1977). The 1939 date seems more likely because mass breeding of a *Ae. aegypti* strain for large-scale experiments was routinely conducted in the Orlando facility in 1942. This strain also was shared with the USDA laboratory in Beltsville, MD (Casanges et al. 1949). Because this strain had no strain designation, strictly speaking, its relationship with the so-called “Orlando” strain is not entirely clear. However, it is highly probable that the two strains were identical because it was directly received from the originating institution (University of Notre Dame 1961).

U.S. Naval Medical Research Institute (NMRI). Research activities using laboratory-bred *Ae. aegypti* at the NMRI were recorded in 1941. The earliest document found reveals that a strain used in 1945 had been derived from the Bureau of Entomology and Plant Quarantine of USDA, Beltsville, MD (Terzian and Stahler 1958). The only strain then available at USDA was the aforementioned “Orlando” strain. It is unknown if the “USNM” strain obtained from the U.S. Navy (Jones and Pilitt 1973) was the same strain from the USDA.

A Strain at the London School of Hygiene and Tropical Medicine (London SHTM). A brief historic review of *Ae. aegypti* breeding in the United Kingdom before 1925 is useful for identifying the strain at the London SHTM. Historically, *Ae. aegypti* eggs were shipped from the tropics (Cuba) to the United Kingdom even before 1912, primarily for systematics and bionomics studies (Howard et al. 1912). In the tradition of the Colonial Office in the early twentieth century, West Africa generally referred to Ghana, Nigeria, and Sierra Leone. In the 1910s, in all three locations there had researchers engaged in *Ae. aegypti*-borne disease investigations in Ghana (Macfie 1915), Nigeria (Seidelin and Summers-Connal 1914), and Sierra Leone (Bacot 1917, 1918). The first strain of West African origin was established in the United Kingdom at the Wellcome Bureau of Scientific Research (WBSR) in 1913 by Malcolm E. MacGregor, with the eggs shipped by Arthur W. Bacot in Sierra Leone (MacGregor 1913). It is unknown how long this strain was maintained, but, it is highly possible that this strain was not the source of a strain established at the London SHTM in 1926.

The first strain established at the London SHTM in 1926 is known to have been derived from West Africa (Lumsden 1947, Bertram et al. 1964, Laurence 1964), but the exact location has never been identified. It is interesting to note that circa 1926 through 1929 Edward Hindle and M. E. MacGregor of the WBSR used a strain of West African origin (location unknown but with a possibility of Liberian origin) obtained from Erich C. W. Martini of Hamburg, Germany (Hindle 1929, MacGregor 1929). That MacGregor was in charge of maintaining this strain from Germany for Hindle in 1926 suggests that the strain from Sierra Leone he had established in 1915 was no longer available by 1926. Had the Sierra Leone strain been still available around 1926, Hindle and MacGregor would probably not have taken the trouble obtaining another strain of West African origin from Germany. Accordingly, the possibility of the London SHTM acquiring the strain from the WBSR in 1926 cannot be ruled out.

Liverpool School of Tropical Medicine (Liverpool STM). The precise origin of the “Liverpool” strain is unclear. An examination of the Annual Reports of the Liverpool STM and associated publications suggests that the date of first maintenance at the School fell between 1935 and 1938 (H. Townson, personal communication). The strain has been described as of West African origin (Macdonald 1962a), with Freetown, Liverpool School of Tropical Medicine (Liverpool STM). The precise origin of the “Liverpool” strain is unclear. An examination of the Annual Reports of the Liverpool STM and associated publications suggests that the date of first maintenance at the School fell between 1935 and 1938 (H. Townson, personal communication). The strain has been described as of West African origin (Macdonald 1962a), with Freetown,
Sierra Leone, the most likely source (W. W. Macdonald, personal communication).

As for the existence of red-eye and black-eye "strains," a clarification is necessary. The original "Liverpool" strain was a composite of ≈30% filaria-susceptible and 70% nonsusceptible mosquitoes. Through a series of single-pair sibling crosses, a highly susceptible black-eye strain ("SS") carrying the filaria-susceptibility allele (P<sub>0</sub>) was selected (Macdonald 1962a,b). After further crossings and backcrosses between the "SS" and a red-eye refractory strain supplied by G. B. Craig (with the red-eye mutant isolated by G.A.H. McClelland), Macdonald and Sheppard (1965) then constructed a strain that is homozygous for both P<sub>0</sub> and the red eye mutation ("re"). This is known as "ref<sub>0</sub>" strain (Macdonald and Sheppard 1965). This strain ("ref<sub>0</sub>") was sent to multiple laboratories in the United States beginning in the 1960s. In some of the receiving laboratories in the United States, for reasons not fully understood but possibly through spontaneous mutation, a black-eye filaria-susceptible strain emerged (H. Townsend, personal communication). Thus, what is known as "black-eye strain" in the United States is technically not the black-eye strain "SS" directly derived from the original "Liverpool" strain and should be clearly distinguished in scientific reporting.

A Global Survey of Laboratory Breeding

This survey of laboratory breeding worldwide before 1945 summarized in Table 1 reveals the extent of research activities with this mosquito in the early part of the twentieth century. The coverage of the institutions in the United States is limited to three.

Mexico and the Caribbean. During the yellow fever investigation in Mexico by the USPHS in 1902–1904, a local Mexican strain was used in a vertical transmission study (Rosenau and Goldberger 1906). At about the same time in Mexico, an expedition team from the Liverpool STM led by Harald Seidelin used a strain for a short period. Regarding the concern of continuing spread of the yellow fever vector to higher ground in Mexico, a strain derived from Vera Cruz was reared in Mexico City. The strain was found to grow and demonstrated human-biting behavior (López 1905). The strain from the Finlay Institute in Cuba was shipped to multiple laboratories in Asia (Fig. 1). Cuba was also the preferred site for an expeditionary group from the

Table 1. Global survey of the use of laboratory-bred *Ae. aegypti*

<table>
<thead>
<tr>
<th>Geographic region</th>
<th>Country</th>
<th>References recording the use of indigenous strains and/or imported strains [&quot;original strain designation&quot; or geographic origin]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central America</td>
<td>Mexico</td>
<td>Indigenous strains: Rosenau and Goldberger 1906, López 1905, Bliss and Gill 1933</td>
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<tr>
<td>South America</td>
<td>Brazil</td>
<td>Indigenous strains: Lowy 1900, Marchoux and Simond 1905, Gordon and Young 1921 and 1922,</td>
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<td>Asia</td>
<td>India</td>
<td>Indigenous strains: Barraud 1928, Rao and Iyengar 1932</td>
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<td>Imported strains: Hoffmann 1927 [&quot;Cuba&quot; strain]</td>
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<td></td>
<td>Indonesia</td>
<td>Indigenous strains: Snijders et al. 1931</td>
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<td>Imported strains: Hoffmann 1927 [&quot;Cuba&quot; strain]</td>
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<tr>
<td></td>
<td>Japan</td>
<td>Imported strains: Miyao et al. 1944, Sasa 1944, 1958</td>
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<td></td>
<td>Malaysia</td>
<td>Imported strains: Hoffmann 1927 [&quot;Cuba&quot; strain]</td>
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<tr>
<td>The Pacific</td>
<td>Vanuatu</td>
<td>Indigenous strains: Ruxton and Hopkins 1927</td>
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<tr>
<td>Africa</td>
<td>Fiji</td>
<td>Indigenous strains: Bahr 1912</td>
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<td></td>
<td>Australia</td>
<td>Indigenous strains: Bancroft 1906, Cleland et al. 1918, Hamlyn-Harris 1928, Heydon 1931</td>
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<td></td>
<td>Ghana</td>
<td>Indigenous strains: Seidelin and Summers-Connal 1914, Stokes et al. 1928a,b, Philip 1929</td>
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<td>Nigeria</td>
<td>Indigenous strains: Macle 1915</td>
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<td>Senegal</td>
<td>Indigenous strains: Mathis 1934a,b; Mathis 1937 [&quot;Dakar&quot; strain]</td>
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<td>Tunisia</td>
<td>Indigenous strains: Pettit et al. 1930</td>
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<td></td>
<td>Sierra Leone</td>
<td>Indigenous strains: Bacot 1917, 1918</td>
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<td>Somalia</td>
<td>Indigenous strains: Drake-Brockman 1913</td>
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<td>Tanzania</td>
<td>Indigenous strains: Roubaud 1937</td>
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<td>South Africa</td>
<td>Indigenous strains: DeMeillon et al. 1945</td>
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<td>Europe</td>
<td>Greece</td>
<td>Indigenous strains: Blanc and Caminopretos 1928</td>
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<td></td>
<td>Denmark</td>
<td>Imported strains: Wigglesworth 1938</td>
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<td></td>
<td>Germany</td>
<td>Imported strains: Hindele 1929 [West Africa], Pagast 1936 [West Africa]</td>
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<td>The Netherlands</td>
<td>Imported strains: Snijders et al. 1931 [Indonesia], Pettit et al. 1930 [Indonesia]</td>
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<td>United Kingdom</td>
<td>Imported strains: Hindele 1929 [West Africa], Howard et al. 1912 [Cuba], Lewis 1933</td>
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<td>[West Africa], MacGregor 1915 [Sierra Leone]</td>
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<td>North America</td>
<td>United States</td>
<td>Indigenous strains: Chandler and Rice 1923, Rozeboom 1939</td>
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<td></td>
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<td>Imported strains: Bliss and Gill 1933 [Cuba]</td>
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The strains maintained for longer periods are shown within double quotation marks. The strains not in quotation marks were mostly maintained only for short periods. The existence of the strains in quotation marks is uncertain due to problems of strain source reporting and other miscellaneous difficulties described in the text. Most of them are presumed to have been lost. The eight strains selected for in-depth review are not included in the table.
United States for obtaining eggs for strain establishment (Bliss and Gill 1933).

South America. In Brazil, Adolfo Lutz used a strain from São Paulo in 1902–1903 to conduct a mosquito-borne transmission study of YFV, by using volunteers (Löwy 1990).

Three European countries (France, Germany, and the United Kingdom) as well as the RFIMR of the United States sent missions to Brazil for YFV studies. Except for the German Mission, all had an *Ae. aegypti* strain ranging from a short period to >10 yr. The French Mission to Brazil used a strain from Rio de Janeiro to conduct a vertical transmission study (Marchoux and Simonet 1905).

A strain derived from Manaus was established before 1922 by the British Mission from Liverpool STM (Gordon and Young 1921, Young 1922). The Yellow Fever Laboratory of the RFIMR in Bahia had a local strain established by Paulo C. B. Antunes. This strain also was used in its laboratory in Rio de Janeiro until the closure of the Bahia laboratory in 1939 (Frobisher et al. 1931, Shannon and Putnam 1934, Whitman 1937, Whitman and Antunes 1938) and was sent to the Institut Pasteur de Dakar in 1933 (Mathis 1937). The RFIMR laboratory in Bahia, Brazil also used the “Athens” strain obtained from Greece (Shannon and Putnam 1934).

In Rio de Janeiro, after 1942, in the joint yellow fever research between Brazilian Ministry of Health and the Rockefeller group, a strain from Nova Iguaçu was used (Waddell and Taylor 1947). In the Fundação Instituto Oswaldo Cruz, the strain was used initially for yellow fever research by Henrique Aragão and was the strain established in Bahia by the RFIMR. A local strain was used in Belo Horizonte in early 1940s for parasitological studies (Paranese 2004).

Asia. In India, S. Rickard Christophers, though absorbed in malaria studies in 1927 in Kasauli, India, still found time to study geographic strain difference of *Ae. aegypti* by examining a strain sent from W. H. Hoffmann of Havana, Cuba (Hoffmann 1927; Fig. 1). From the perspective of his professional career development, this work could be one of the ground works on this species, because he would publish a monumental treatise on *Ae. aegypti* 33 yr later at the age of 87. Sometime before 1928, a strain from Punjab established by Philip J. Barraud was shipped to the United Kingdom and West Africa (Barraud 1928, Hindle 1929). A strain from Bengal was used in experimental infection with *Wuchereria bancrofti* (Rao and Iyengar 1932). The “Assam” strain was used in a comparative susceptibility test to *Dirofilaria immitis* infection (Roubaud 1937).

In Indonesia, S. L. Brug of Batavia (now Jakarta) had a colony that was used in Indonesia and also shipped to Cuba for a comparative taxonomic study with a Caribbean strain. In turn, Brug received a strain from Havana, Cuba (Hoffmann 1927; Fig. 1). The strain established in Batavia by Brug also was shipped and maintained at the Military Hygiene Laboratory in Weltevreden, the Netherlands (Pettit et al. 1930). A strain established in 1930 in Medan, Sumatra was extensively tested for DENV transmission by a group of physicians from the Netherlands (Snijders et al. 1931). In Japan, during WWIII, three strains derived from New Britain, New Guinea, and the Philippines were established (Miyao et al. 1944; Sasa 1944, 1958). They were used for transmission of the avian malarial parasite *Plasmodium gallinaceum*.

In Malaysia, A. T. Stanton of the Institute of Medical Research, Kuala Lumpur, received a strain from Hanoi (Hoffmann 1927; Fig. 1). The origin of a local strain used by Vincent B. Wigglesworth for an osmoregulation study during his visit to Malaysia is unknown. In the Philippines, a local strain was established in 1925 by Siler. In Vietnam, a strain each was established in Saigon (now Ho Chi Minh City) and in Hanoi by the Institut Pasteur (Toumanoff 1937). The strain from the Tonkin area of Vietnam was used by the French Mission for determining the possibility of *Ae. aegypti* hybridization with *Ae. albopictus* (Toumanoff 1937, 1938, 1950), whereas the strain from Saigon was used by Roubaud in Paris, France (Toumanoff 1937).

The Pacific. Strains were established in Samoa and New Hebrides (currently Vanuatu) for a bionomics study (Buxton and Hopkins 1927). In Fiji, a local strain was used for a filarial infection study (Bahr 1912).

Australia. In Queensland, Thomas L. Bancroft used a colony to prove for the first time in history that *Ae. aegypti* was the true vector of DENV (Bancroft 1906). Other strains from Brisbane, Queensland were used for DENV transmission (Cleland et al. 1918, Hamlyn-Harris 1928). Another strain was used primarily as negative control for a study of transmission of *W. bancrofti* parasite (Heydon 1931).

Africa. A strain was established in Accra, Ghana, as early as 1915 (Macfie 1915). In Nigeria, the first strain was established in Yaba (near Lagos) in 1914 by the British West Africa Yellow Fever Commission (Seidelin and Summers-Connaught 1914). The Rockefeller Mission to West Africa also had a local *Ae. aegypti* strain in 1928 for the study of the Asibi strain of YFV (Stokes et al. 1928a; Philip 1929). In Senegal, the French Mission had a strain in Dakar. This strain was used to feed on yellow fever patients and to transmit the YFV to rhesus monkeys (Mathis 1934a, b). It is not entirely clear if this strain and the “Dakar” strain used by Mathis in Senegal and Roubaud in Paris were identical (Mathis 1937, Roubaud 1937). Additional strains at the Institut Pasteur de Dakar included the “Athens” strain from Greece, “Java” strain from Indonesia, and “Bahia” strain from Brazil (Mathis 1937).

In Somalia and Tunisia, a strain was established by B. E. Drake-Brockman and by M. Colas-Belour, respectively (Drake-Brockman 1913, Pettit et al. 1930). In Tanzania, a strain was established circa 1937 (Roubaud 1937), but the exact source information was not found. In South Africa, one strain was established from Salisbury Island near Durban (DeMeillon et al. 1945).

Europe. The original source information of the “Athens” strain is not known. However, it is probably identical to the strain established there in 1928 at the Institute Pasteur d’Athènes in the wake of a major
dengue outbreak. The Institut Pasteur in Athens was the only institution engaged in laboratory research involving *Ae. aegypti* in Greece at that time (Blanc and Caminopteros 1928). This strain has a historical significance, because the vector mysteriously disappeared from the eastern Mediterranean areas thereafter even without an organized vector control campaign (Curtin 1967). The strain was sent to the Institut Pasteur de Dakar, Senegal (Mathis 1937) and to the Rockefeller laboratory in Bahia, Brazil (Shannon and Putnam 1934).

In Denmark, the source of the strain used by V. B. Wigglesworth at the University of Copenhagen (Wigglesworth 1938) was probably the strain maintained at the London SHTM since 1926. In France, Institut Pasteur in Paris coordinated medical entomological research with its numerous branch institutions overseas, where *Ae. aegypti* was investigated (Opinel 2008). Because of the availability of multiple strains of this mosquito, the French were, like the British, well ahead of other nations in recognizing the importance of strain difference. Thus, Emile Roubaud in Paris used in one study as many as four strains (“As-sam” from India, “Tanganyka” from Tanzania, “Cuba” from Havana, Cuba, and “Dakar” from Senegal; Roubaud 1937, Mathis 1938). This “Dakar” strain used in 1930s in Dakar and Paris was not the “Dakar” strain used more recently (Turell et al. 1992). Mathis also used the “Athens” strain from Greece, “Java” strain from Indonesia, and “Dakar” strain and “Bahia” strain from Brazil (Mathis 1937).

In Germany, E.C.W. Martini of the Maritime and Tropical Medicine Institute in Hamburg (renamed after 1942 as Bernhard Nocht Institute of Tropical Medicine) had a strain of West African origin for an unknown period before 1926 (Hindle 1929). Although the source information could not be found, Monrovia, Liberia is strongly suspected to be the origin of this strain. During an outbreak of yellow fever in 1925 in Monrovia, Liberia, at the direction of Martini, W. O. Wehrle investigated the clinical syndrome of patients and vector infestation (Wehrle 1928). If a strain was then established in Hamburg, the 1925 date would reasonably agree with the unspecified date retrospectively deduced from the account of Hindle (Hindle 1929). As mentioned earlier, a strain was sent from Martini to WBWR in the United Kingdom around 1926. The same strain also was sent from Hamburg to the University of München (Pagast 1936). In the Netherlands, the strain from Sumatra established by the aforementioned group from the Netherlands was used in Amsterdam for human experiments involving DENV and for mouse experiments with YFV (Snijders et al. 1931). Another strain established in Java by Brug was maintained at the military hygiene laboratory in the Netherlands (Petitt et al. 1930). It is highly likely that this was the “Java” strain used in Senegal by Mathis (Mathis 1937).

In the United Kingdom, E. Hindle of the WBSR used the aforementioned strain of West African origin as well as a strain obtained from P. J. Barraud in Punjab, India, sometime before 1926 in vector competence studies with YFV (Hindle 1929). Frederick V. Theobald of South-Eastern Agricultural College received eggs in early 1910 or so from Cuba for his systematics and bionomics studies (Howard et al. 1912), whereas D. J. Lewis of the British Museum (Natural History) used the “London” strain from the London SHTM (Lewis 1933).

**United States.** During the dengue outbreak in Texas in 1922, a local strain was established and used for transmission experiments, by using volunteers (Chandler and Rice 1923). A local strain was also established in Stillwater, OK (Rozeboom 1939). A team of researchers from the University of Tennessee visited Cuba in 1933 and returned home with eggs to establish a strain (Bliss and Gill 1933).

**Selection of Location for Obtaining Eggs and Maintenance of Multiple Strains per Institution**

This review revealed that as far as major institutions in the continental parts of the United States before 1938 are concerned, sources for *Ae. aegypti* for laboratory colonization were in the former Spanish territories (Cuba, Philippines, and Puerto Rico) acquired after the Spanish-American War of 1898 and in Nigeria rather than from much closer locations at home in the southern states where distribution of this mosquito and sporadic yellow fever and dengue outbreaks derived from imported cases were well known (Kumm 1931). Probably the most important reason for this was mosquitoes with optimal qualities for mosquito-borne parasites, microorganisms, and viruses. The four locations were probably assumed highly suitable because each was endemic for yellow fever or dengue or there were prior records of laboratory transmission experiments conducted earlier using mosquitoes from these areas.

For European countries, the reason for selection of West Africa was probably due to convenience due to geographic proximity and to historic evidence of continual yellow fever transmission over several centuries. Establishment of a strain from Greece was the result of the 1928 outbreak of dengue with >1,000 fatal cases. For a smaller number of investigators in Europe, however, securing multiple strains each representing a different region of the tropics was their major interest because they recognized the importance of comparative studies among geographic strains. Soon thereafter, the importance of strain variation was also recognized in the United States, leading to a trend of multistrain maintenance in many institutions.

**Applications of Laboratory Strains in the Early History of *Ae. aegypti***

**Systematics and Bionomics.** Eggs were shipped from the tropics to entomologists in temperate regions as well as in other parts of the tropics for morphological, systematics and bionomics studies early in the twentieth century (Howard et al. 1912, Hoffmann 1927). As early as in 1902, a comprehensive treatise of *Ae. aegypti* bionomics was available (Agramonte...
YFV in Asia despite the ubiquitous infestation by Ae. aegypti (Hoffmann 1927, Dudley 1934). Atypical nocturnal feeding activity also was studied (Marchoux and Simond 1905, Gordon and Young 1921).

Physiology and Rearing Methods. The mechanisms involved in egg hatching were one of the major interests in the early history of Ae. aegypti studies, because inconsistency of egg hatching was a major impediment in research. The early discovery of the association of the growth of microorganisms with increased frequency of hatching in 1915 (Bacot 1917) was followed by a number of publications (Young 1922, Buxton and Hopkins 1927, Roubaud and Colas-Belcour 1927, Roubaud 1929, Mathis 1934b, Shannon and Putnam 1934, Rozeboom 1935). This ultimately led to the understanding of reduced oxygen as a stimulant (Gjullin et al. 1941). A fortuitous discovery that the eggs in an envelope inadvertently left unattended for nearly 3.5 mo were still viable led to more efficient and simpler preservation of Ae. aegypti strains (MacGregor 1915). The functional role of anal papillae for osmoregulation was determined by Wigglesworth (1938).

Both larval and adult nutritious requirements were the interest of many researchers to improve rearing (MacGregor 1915). Artificial blood feeding of adult females and the relation of bloodmeal size and ovulation were studied (Roy 1936), whereas the effect of larval nutrition was studied in relation to fecundity (Mathis 1938). Strain variation in fecundity was studied by genetic crossing among the strains from South America, Asia, Greece, and Africa (Mathis 1937).

Pledge feeding using skin of a bat or of a rat was developed as early as 1922 (Bishop and Gilchrist 1946). Axenic (microorganism-free) culture of larvae was also attempted to determine the roles of microorganisms in larval development (MacGregor 1929, Trager 1935). By 1941, many factors modulating growth, fecundity, and feeding activity were determined under controlled environmental conditions (temperature and light) (Seaton and Lumsden 1941). Improvement in equipment (such as pupal separators, adult transfer devices, and special trays), temperature and humidity control, and other features in facility design made efficient mass rearing a routine procedure (Leeson 1932, Lewis 1933, Johnson 1937).

Replication of Microorganisms and Viruses, Transmission Studies, and Vaccine Development. One of the initial interests in the use of Ae. aegypti strains was for studying replication and life stages of helminthic protozoan parasites, such as Brugia malayi, D. immitis, Plasmodium gallinaeum, and Plasmodium lophurae (Coggershall 1938, Laird 1941, Trager 1942). Roubaud (1937) used four strains (“Assum,” “Tanga nyka,” “Dakar,” and “Cuba”) to study genetic difference in susceptibility to infection with D. immitis. Uses of the mosquito strains for YFV studies were extensive. Strains were used for biological transmission of YFV in Havana, Cuba, by the Walter Reed Commission and of DENV in Amsterdam (Dinger et al. 1930). The other major subjects studied were extrinsic incubation period (Bauer and Hudson 1928, Davis 1932, Sellards 1935), longevity of infected mosquitoes (Beeuwkes et al. 1933), dynamics of replication of virus (Whitman 1937), and vector competence or refractoriness (Hindle 1929, Dinger et al. 1930), and Pettit et al. (1930) used multiple strains and proved that Asian strains were permissive to YFV replication. The conclusion of negligible probability of transmission of the attenuated 17D vaccine by the infected mosquitoes to rhesus monkeys (Whitman 1939) provided the critical information needed before the commencement of a massive YFV vaccination campaign in the Americas.

Mosquito infection in the larval stage was also studied (Whitman and Antunes 1938). Vertical transmission of YFV or DENV in mosquitoes was another popular subject (Reed et al. 1901; Marchoux and Simond 1905; Rosenau and Goldberger 1906; Seidelin and Summers-Connal 1914; Siler et al. 1925; Stokes et al. 1928a,b; Davis and Shannon 1930; Frobisher et al. 1931; Simmons et al. 1931a, Hinman 1933). Although nearly all results were negative, the only positive result by Marchoux and Simond (1905) was reconfirmed >90 yr later (Fontenille et al. 1997). The possibility of venereal transmission of DENV between Ae. aegypti males and females was investigated in the Philippines (Simmons et al. 1931b).

Although it was known that Ae. aegypti was not a natural vector, nonetheless, a strain was used to confirm replication of eastern equine encephalitis virus and western equine encephalitis virus (Merrill et al. 1934). For the studies of herpes virus and lymphocytic choriomeningitis virus, Ae. aegypti strains were used primarily to rule out the possibility of vector-borne mode of transmission (Simmons et al. 1933, Coggershall 1939). The results of experimental infection with bacterial pathogens Leptospira icterohaemorrhagiae and Francisella tularensis were negative (Gay and Sellards 1927, Philip et al. 1932).

Three protocols were used for attenuation of dengue virulence for a vaccine development: repeated cycles of human–mosquito passage, repeated passage in mosquitoes, and X-ray irradiation of infective mosquitoes (Siler et al. 1926, Holt et al. 1931, Simmons et al. 1931a).

Mosquito Control. Beginning in 1942, a strain available at the Orlando, FL facility of the USDA was used extensively for experiments to determine the efficacy of numerous insecticides (including DDT) and repellents. New insecticide application techniques, including ultralow-volume application, were developed during WWII (McDuffe 1963). By 1948, insecticide resistance in mosquitoes was detected in Florida, necessitating in subsequent experiments the use of a nonresistant strain, such as the “ROCK” as a reference. In both Orlando, FL, and United Kingdom, Ae. aegypti was found to be valuable in standardized tests because
the mosquitoes could be mass produced relatively easily under identical conditions (Christophers 1945).

Hybridization. The possibility of hybridization between *Ae. aegypti* and *Ae. albopictus* was examined in Vietnam. The sole positive result obtained by Constantin Toumanoff of the Institut Pasteur (1937, 1938) could not be reproduced by all other researchers.

Biosafety. In the early part of the history of work with *Ae. aegypti*, strict regulations pertaining to the shipment of infected arthropods or other animals comparable with the required standards today, did not exist. But, the main reason why mosquitoes infected with DENV or YFV were shipped from Indonesia to Amsterdam or from Brazil to Paris for experiments (Snijders et al. 1931, Harvey 1979) was that there was no means to otherwise preserve virus during a long journey (as long as a few weeks depending on route) by ship. Transportation of infected mosquitoes was the most reliable method. Only in 1928 was it learned that infectivity of YFV could be preserved for at least 12 d by freezing (Harvey 1979). The unusual arrangement of shipping infected mosquitoes for experiments outside the tropics was adopted to conduct hazardous research safely at cooler, vector-free locations to prevent accidental spread of disease by escaped mosquitoes. The same precautionary measure was taken in the United States during the mass production of yellow fever vaccine in the 1940s, by conducting all production and related experiments with the vector only at the RML in Montana where overwintering of escaped tropical vectors was impossible. In United Kingdom, too, at the urging of the Ministry of Health, the YFV investigation of E. Hindle was moved from the laboratories maintaining multiple strains may have contributed to heterogeneity (Gooding 1966, Ahmadi and McClelland 1985). For example, 6 yr after the strain (later called “ROCK”) was received at the Rutgers University from the RFIMR-Princeton (Fig. 1), another strain obtained from the USDA (later called “Orlando” strain) was mixed to strengthen the former strain (Sutherland 1964). The “Orlando” strain itself had been regularly supplemented with field mosquitoes (location unidentified) until circa 1992 (Williams et al. 2006). Assuming that the “field mosquitoes” used to supplement were obtained locally in Florida, the significance in terms of homogeneity of the strain is that the original strain was insecticide-susceptible (which is why it was used in the aforementioned insecticide efficacy studies in 1942) but the supplements after 1950 were probably insecticide resistant. Apparently, alteration in mosquito population composition was a deliberate strategy sometimes adopted “to prevent loss of field characteristics and genetic variability” in many laboratory strains (Munstermann 1994).

Laboratory strains maintained for a long period often show loss of original traits or acquisition of characteristics atypical of the wild strain and may become unsuitable for laboratory experiments depending on the research objective (Lorenz et al. 1984, Armstrong and Rico-Hesse 2001, Scott et al. 2006, Grieco et al. 2007). Another cause for a change in the characteristics of a strain is the loss of the strain and replacement with a strain of the same designation from another laboratory. Thus, depending on the history of strain maintenance including from which institution and when a strain in question was obtained, as well as on past history of attempt to artificially alter original strain, authenticity of the same strain used by multiple laboratories should not be assumed automatically. Accidental mislabeling of a strain in a laboratory is another cause of confusion. For these reasons, periodic strain identification for reconfirmation using strain-specific sequences (preferably in multiple loci) using molecular mosquito identification tools is highly desirable.

Problems Related to Strain Designation and Strain Source Identification in Scientific Publications

In this review, multiple problems of strain source identification in the early literature were found. A survey of literature for the entire history of research with *Ae. aegypti* conducted during this review confirmed that these problems were not limited to the early history but persist even today. In the following discussion summarizing the problems, all institutions originating a strain are called originating institutions. All institutions which did not originate a strain but which received it either directly from an originating institution or indirectly from another institution are called receiving institutions. All institutions (including originating institutions) that shipped eggs to other institutions are called donor institutions. Thus, any receiving institution also became a donor institution, if it shipped eggs to other institution(s) upon request.

No Documentation of Strain Establishment. Before 1938, because establishment of a strain was a novelty, it was frequently documented in publications either by the originating or receiving institutions. In contrast, after 1938, strain establishment was very rarely documented by originating institutions because it was no longer publishable by itself due to loss of novelty. The information pertaining to the origin of a strain was
sporadically described only when some of the originating or receiving institutions used it in their reports using the strain.

The consequence of this unfortunate tradition over many years was the accumulation of many undocumented strains, some of which were much later recognized to be of historical importance or highly useful because of the possession of unique characteristics valuable for current research. Here are a few such examples. The “Bora Bora” strain, has been used recently as the World Health Organization (WHO) standard in insecticide resistance studies of *Ae. aegypti* populations (Thiery et al. 1999, Macoris et al. 2005). This undocumented strain was originally established by Gaston Pichon of Institut de Recherches pour le Développement, Bondy, France from the mosquitoes collected in Bora Bora of the French Polynesia in the 1960s (P. F. Guillet, personal communication). The “UGAL” strain (abbreviation for the University of Georgia Laboratory) popularly used in molecular genetic studies today is one of the undocumented strains that was probably established around early 1970s using the local mosquitoes in Georgia (Klowden and Lea 1978). The parental strains used to obtain the white eye mutant was established at the University of Notre Dame (Bhalla 1968) and was later used to generate the first transgenic mosquito strain (Jasinskiene et al. 1998). The parental strain was “New Texas” or “Texas” (A. Mori, personal communication; Craig 1965b) that was probably derived from a wild strain designated “New Mick” established in late 1950s by Don W. Micks of the University of Texas, Galveston, TX, because the designation “Texas” was interchangeably used by Craig for “Mick” (Craig 1965b).

Although the importance of documenting correct information for strains may not be apparent at the time of establishment, the significance may arise much later unexpectedly. Unfortunately, it can later become extremely difficult or impossible to obtain the original strain source information because unpublished records were lost and/or knowledgeable individuals cannot be contacted.

**Problems at Strain-Receiving Institutions.** In many publications, a strain kept at the author’s institution was identified as the source. In the others, donor institutions were identified as sources. Usually, neither were the originating institutions. In the latter cases, in particular, if the donor institutions had multiple strains, the practice of identifying strain source by the donor’s institutional name or by geographic location resulted in multiple strain names for the same strain (such as “Orlando” strain) (Craig et al. 1961), or the same name (or very similar names) adopted for multiple, distinct strains (such as “Bangkok” and “Puerto Rico [or PR’]”). A new type of strain designation in the past few decades has been joint institutional names (such as “NIH-Rockefeller” and “UGAL-Rockefeller”) whose original strain source data could not be found in published documents. Thus, it is unknown if they represent mixtures of two strains (“NIH” or “UGAL” and “Rockefeller”), “Rockefeller” strain obtained from NIH or UGAL, or mismomers. Because no record of strain mixing has been found for them, either the second or third possibility seems to be most likely. These strain designations are confusing to those who are familiar with the traditional designations. Collectively, these problems make it difficult to trace the strain origins in the literature.

**Relevance of the Importance of the Past Problems in Current Research.** In the early part of the history of *Ae. aegypti* research, problems were mostly a matter of individual style of scientific writing and the lack of standards. Later, in the modern period, standards and styles for scientific publications have become more firmly established, and disclosure of the sources of materials, methods, and other relevant information has often been required to ensure reproducibility. It is ironic that, today, original strain source information cannot be depended on publications. Often researchers have to rely heavily on unconventional methods, such as communications with potentially knowledgeable individuals or originating institutions and search through unpublished archival records. Similar problems affecting mosquito systematics for museum specimens have been noted previously (Foley et al. 2005).

The major root cause of problems concerning *Ae. aegypti* strain designation and source identification in publications is the absence of a convenient repository of strain source information in the public domain for strain originators, and the absence of a universal system of strain identification in publications. An easy online registration of mosquito strains (current and new) is one way to encourage deposition of strain source information in the public domain. A system already developed in biotechnology for securing a unique identification number for each sequence upon deposition as prerequisite for submission of scientific articles and mandatory use of unique identification number in all scientific documents are useful models. For *Ae. aegypti* strain deposition, expansion of the existing VectorBase for molecular data with a capability of online cross-indexing to accommodate a set of biological and other characteristics of strains seems to be one of the useful approaches to be considered. The recognition of the importance of mosquitoes to human health has increased research efforts to many species of mosquitoes. Similar issues addressed here for *Ae. aegypti* apply to these other mosquito species.

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References Cited


Ahmadi, A., and G. A. H. McClelland. 1985. Which this study was not possible.

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Bacot, A. W. 1918. A note on the period during which the eggs of Stegomyia fasciata (Aedes calopus) from Sierra Leone stock retain their vitality in a humid atmosphere. Parasitology 10: 280–283.


Bancroft, T. L. 1906.


Christophers, R. 1945.


a view to applying this method to the chemotherapy of malaria. Parasitology 37: 85–100.


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Hoffmann, W. H. 1942. Letter from W.H. Hoffmann to George A. Kellogg, March 2, 1942. Philip S. Hench Walter Reed Yellow Fever Collection, University of Virginia, Charlottesville, VA.


Thompson, C. 1938. New views on the nature of the intercroisement de *St. albopictus* Skuse with *St. argentea*. J. Econ. Entomol. 39: 627–630.


Tumanski, G. 1938. New views on the nature of the intercroisement de *St. albopicta* Skuse with *St. argentea*. J. Econ. Entomol. 39: 627–630.


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