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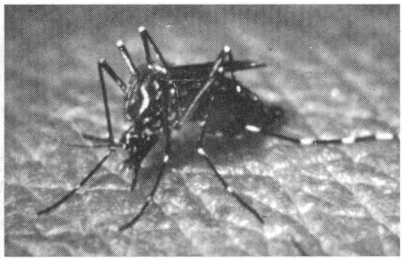

The Transmission of Dengue Fever in Puerto Rico: An Epidemiologic Approach Using a Geographic Information System

U.S. GEOLOGICAL SURVEY
Water-Resources Investigations Report 98-4119

U.S. Department of the Interior
U.S. Geological Survey

Cover Photo

Photograph of *Aedes aegypti* mosquito courtesy of the National Center for Infectious Diseases, Centers for Disease Control.

Photograph of mogotes in the municipio of Florida, Puerto Rico (photo by Amy C. Morrison).

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By Amy C. Morrison, Marilyn Santiago, José G. Rigau-Pérez, and Paul Reiter

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San Juan, Puerto Rico
1998

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Thomas J. Casadevall, Acting Director

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For additional information write to:

District Chief
U.S. Geological Survey
GSA Center, Suite 400-15
651 Federal Drive
Guaynabo, Puerto Rico 00965-5703

Copies of this report can be purchased from:

U.S. Geological Survey
Branch of Information Services
Box 25286
Denver, CO 80225-0286

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CONVERSION FACTORS, ACRONYMS, AND ABBREVIATIONS

Multiply	By	To obtain
centimeter (cm)	0.3937	inch
kilometer (km)	0.6214	mile
meter (m)	3.281	foot
millimeter (mm)	0.03937	inch
square kilometer (km^2)	0.3861	square mile

Temperature: Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows: °C = 5/9 x (°F - 32)

ACRONYMS AND ABBREVIATIONS

Ae. aegypti	Aedes aegypti
AML	Arc macro language
CDC	Centers for Disease Control and Prevention
C6/36	Mosquito cell (Aedes albopidus) tissue culture line
CDT	Centro de Diagnóstico y Tratamiento
DCI	Dengue case investigation
DHF	Dengue hemorrhagic fever
DLG	Digital line graph
DSS	Dengue shock syndrome
EBV	Ebstein-Barr virus
EIP	Extrinsic incubation period
ELISA	enzyme-linked immunosorbent assay
GIS	Geographic information system
GPS	Geopositioning system
HAFI	División Higienización Ambiente Físico Inmediato
HI	Hemagglutination-inhibition
HIV	Human immunodeficiency virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MAC-ELISA	IgM antibody-capture enzyme-linked immunosorbent assay
PRDOH	Puerto Rico Department of Health
RNA	ribonucleic acid
U.S.	United States
USGS	U.S. Geological Survey
TIGER	Topologically integrated geographic encoding and referencing

The Transmission of Dengue Fever in Puerto Rico: An Epidemiologic Approach Using a Geographic Information System

By **Amy C. Morrison[1], Marilyn Santiago[2], José G. Rigau-Pérez[3] *and* Paul Reiter[3]**

Abstract

Dengue fever, a viral disease transmitted by the mosquito *Aedes aegypti*, can spread rapidly in explosive epidemics. In Puerto Rico, dengue is a seasonal disease estimated to be responsible for more than 1,000 hospitalizations annually. Dengue transmission is influenced by the behavior of *Aedes aegypti*, movement and distribution of humans, and virus development within the mosquito. The role of each of these factors is poorly understood, in part, because of the absence of studies on the spatial-temporal patterns of dengue cases. Furthermore, recent failures of mosquito control programs designed to reduce dengue transmission indicate that long standing assumptions about the dispersal of dengue virus by mosquitoes are incorrect. The spatial and temporal distribution of dengue cases reported to the Centers for Disease Control's dengue surveillance system during a 1991-92 outbreak in Florida, Puerto Rico, and a normal transmission season (May 1994 - June 1995) in Ponce, Puerto Rico, were studied using a geographic information system. The two municipalities differed in area, population, climate, and dengue transmission intensity. Dengue cases reported in each of these municipalities were georeferenced by their residential address on Puerto Rico Planning Board digital zoning and U.S. Geological Survey topographic maps. To provide a geographic component to the existing dengue surveillance program in Puerto Rico, weekly case maps were generated for each transmission season, and then the spatial and temporal clustering patterns of the cases were described with a newly developed method called "nearest case pair analysis." For the Florida data, a sophisticated series of exploratory statistical procedures (Barton and David test, K-function analysis, Knox test) were used to describe the observed pattern of spread and case clustering. In addition, the occurrence of three individual dengue serotypes (dengue-1, dengue-2, and dengue-4) were plotted on monthly maps for the five dengue seasons (June-May) beginning in June 1989.

The evolution of the epidemic in Florida was very rapid, affecting a large geographic area within 7 weeks of the first reported case of the season. The Barton and David test identified 23 temporal clusters of cases that had a similar spatial distribution indicating that cases were widely distributed early in the course of the epidemic. Significant dengue case clustering was identified within individual households over short periods of time (3 days or less) but, in general, the cases had spatial pattern characteristics much like the population pattern as a whole. In contrast, the

[1] Centers for Disease Control and Prevention. Now at the University of California, Davis.
[2] U.S. Geological Survey
[3] Centers for Disease Control and Prevention

progression of dengue through Ponce was characterized by the sporadic occurrence of cases for 4 months, after which dengue incidence showed a normal seasonal increase. After the seasonal increase in Ponce, the observed pattern of dengue cases was similar to that of Florida, only on a larger scale. There was less case clustering inside houses in Ponce than in Florida; only 7.3 percent of the houses in Ponce had multiple reports of dengue cases compared with 25.8 percent in Florida.

Although clustering of dengue cases could not be identified beyond closely related cases within households, the rapid temporal and spatial progress of dengue within the community indicate that control measures should be applied simultaneously to the entire municipality, rather than in areas immediately surrounding houses of reported cases.

At the macrogeographic level, three dengue serotypes were circulating in Puerto Rico between June 1988 and May 1994, but the relative abundance of each serotype varied with transmission season. Dengue-4 predominated in 1988, changing to dengue-2 by 1991. During the 1991-92 season, dengue-4 became very uncommon but dengue-1 reemerged and was predominant by 1993.

The spatial analyses carried out during this project confirmed the value of geo-referencing data and the potential value of spatial statistical analyses for defining the spatial scale at which dengue surveillance, prevention, and control should be conducted. This report illustrates how medical entomologists and dengue epidemiologists can improve spatial data collection. The authors conclude that without an accurate address georeferencing system in place, incorporation of dengue case data (residential addresses of reported dengue cases reported) will do little to enhance the current dengue surveillance program in Puerto Rico.

INTRODUCTION

Dengue fever is a mosquito-borne disease caused by one of four closely related **RNA virus** serotypes (dengue-1, 2, 3, 4) belonging to the genus *Flavivirus*, Family Flaviridae (Gubler, 1992). All four **serotypes** infect humans and cause a range of responses that include: **inapparent** and mild **infections**, classic dengue fever (acute febrile illness with headache, body aches, and rash), and the potentially lethal syndrome, dengue hemorrhagic fever (DHF) with shock (dengue shock syndrome, DSS) (Waterman and Gubler, 1989). Infection with dengue virus results in life-long immunity against the infecting serotype; thus, in areas where all four dengue serotypes are circulating, persons can have as many as four dengue infections, one with each serotype (Gubler, 1992). Infection, especially in the young, may produce few or no symptoms. Symptomatic cases of dengue are, therefore, usually a minority of all dengue infections in a community.

Dengue is the most significant arboviral disease affecting humans in the world today, afflicting more than 1 million people per year worldwide (Gubler, 1989b). In Asian countries where dengue is **endemic**, attack rates of the disease are highest in children; if undiagnosed and untreated, case fatality rates can be as high as 30 to 40 percent (World Heath Organization, 1986). There is considerable concern that the natural history of the dengue **epidemic** patterns observed in Asia 20 to 30 years ago is repeating itself in the Western Hemisphere, putting human populations in these areas at risk for severe dengue, DHF, and DSS epidemics (Halstead, 1981; Gubler, 1987; Hayes and Gubler, 1992; Gubler and Trent, 1994).

In Puerto Rico, dengue incidence is fourth on the list of reportable diseases (275 cases per 100,000 population in 1991) and is estimated to be responsible for more than 1,000 hospitalizations annually (Centers for Disease Control and Prevention, 1993). Moreover, the costs attributable to dengue (medical, lost work, and **vector** control) were estimated at $100-150 million (U.S.) for the period 1977-88 (Von Allmen and others, 1979; Gubler, 1989a). In 1994, Puerto Rico experienced its most severe dengue epidemic in 25 years; over 20,000 cases were reported with approximately 10,000 hospitalizations and 13 confirmed fatalities.

Dengue viruses are transmitted from person to person through the bite of an infected mosquito. Dengue transmission is influenced by the abundance, survival rate, and behavior of the principal mosquito vector, *Aedes (Stegomyia) aegypti* (L.), **herd**

immunity to the circulating virus serotype, the density, distribution and movement of humans, and the developmental time of the virus within the mosquito (Gubler, 1992). The relative influence of each of these factors on the dynamics of disease transmission is poorly understood, in part, because of the scarcity of studies focusing on the spatial-temporal patterns of dengue cases. The limited availability of reliable dengue data sets that include sufficient information of date of onset of symptoms and geographic locations of cases account for the absence of spatial studies. Since the immature stages of *Ae. aegypti* develop in water in artificial and natural (for example, coconut shells, bromeliads) containers around houses, environmental conditions directly affect mosquito abundance and survival. Laboratory studies have shown that viral multiplication increases with temperature. Thus, the time required for the mosquito to become infective would decrease with temperature (Watts and others, 1987; Gubler, 1992). Despite the apparent importance of rainfall, temperature, and relative humidity to dengue virus transmission, a consistent correlation of dengue incidence with these factors has never been demonstrated.

Control of dengue depends on reducing the abundance of *Ae. aegypti,* because no vaccines or chemotherapy are available. Two approaches to mosquito control have been employed: elimination or treatment of larval habitats to prevent production of adults, and insecticidal space spraying to reduce adult populations (Gubler, 1992). Most dengue control programs continue to focus their mosquito control efforts around the houses of reported cases of disease; the potential effectiveness of this approach relies on early detection of dengue cases and the assumption that *Ae. aegypti* rarely travel further than 50 to 100 m during their lifetime (World Health Organization, 1986). In the Caribbean, dengue often spreads explosively (appears simultaneously) over large urban areas and focal spraying and other area limited responses have been ineffective in controlling the disease (Gubler, 1989b; Newton and Reiter, 1992). These control failures illustrate the need to better understand the spatial-temporal patterns of the disease.

Purpose and Scope

In an effort to add a geographic component to the existing Puerto Rico dengue surveillance program, a study funded cooperatively by the U.S. Geological Survey (USGS), the Centers for Disease Control and Prevention (CDC), and the Puerto Rico Department of Health (PRDOH) was begun in June 1994. The objectives of this collaboration were to (1) Plot the distribution of reported dengue cases within two Puerto Rico municipalities, with distinct disease transmission patterns, (2) Plot the movement of dengue serotypes over the island for a 5-year period, and (3) Correlate meteorological parameters (temperature and rainfall) to dengue incidence throughout the island.

These studies were possible because of the availability of data from the laboratory-based dengue surveillance system of the San Juan Laboratories, Dengue Branch, Division of Vector-Borne Infectious Disease, National Center for Infectious Diseases, Centers for Disease Control and Prevention (Gubler and Casta-Vélez, 1991). The primary purpose of surveillance is to provide an early warning for epidemic dengue and to monitor disease activity in the community (Gubler, 1989b). Surveillance data is used by dengue prevention and control programs to decide where and when to apply vector control measures. Thus, the overall objective of this project was to use a geographic information system (GIS) to study the temporal and spatial occurrence of dengue in Puerto Rico and to determine if this methodology could be utilized to improve surveillance, prevention, and control of dengue fever.

This report describes the spatial and temporal distribution of reported dengue cases at a local level (microgeographic) during a 1991-92 epidemic in the municipality of Florida and a season of endemic transmission (1994-95) in the municipality of Ponce. The weekly spread of dengue was plotted on maps of reported dengue cases. For the Florida data, a sophisticated series of exploratory statistical procedures were used to (1) Determine if there were any changes in the spatial distribution of reported dengue cases over the course of the epidemic, and (2) Identify both spatial and space-time clusters of disease.

Since a goal of the Puerto Rico dengue surveillance program is to describe long-term trends in dengue activity across the island (macrogeographic), the temporal and geographic occurrence of individual dengue serotypes isolated in Puerto Rico during the five dengue seasons beginning in June 1989 were plotted on maps. To determine if broad climatic differences were correlated with differences in dengue incidence rates, an attempt was made to set the boundaries of climatic zones, defined by average rainfall and temperature, but within municipal boundaries. Finally, this report discusses the utility of incorporating GIS into the current dengue surveillance system used in Puerto Rico.

Previous Studies

Reports of case clusters inside the same or adjacent houses and descriptions of the focal nature of dengue are relatively common (Halstead and others, 1969; Ehrenkranz and others, 1971; Waterman and others, 1985; Gubler, 1992), but studies focusing on the spatial-temporal patterns of dengue cases have been rare and anecdotal. Halstead and others (1969) noted that the onset dates of family members hospitalized with DHF were often separated by only a few days. Waterman and others (1985) demonstrated statistically significant household clustering, but did not assess the time-space relation between these cases.

During the present study, the transmission cycle of dengue, the biological characteristics of *Ae. aegypti* and the previous efforts to examine the link between environmental factors and mosquito abundance are described. Second, the description of dengue case patterns are presented. Finally, background information on geographic studies of infectious disease, especially those that have applied GIS technology, is discussed.

Dengue Transmission Cycle, Disease Seasonality, and *Aedes aegypti* Biology

The temporal distribution of dengue cases is influenced by the transmission cycle of the virus. Onset of symptoms occurs after an **intrinsic incubation period** of 5 to 8 days (range 3-15 days). **Viremia** precedes the onset of symptoms by about a day and lasts 3 to 4 days (Waterman and Gubler, 1989). After biting a viremic individual, a female *Ae. aegypti* mosquito will become infective after an **extrinsic incubation period** (EIP) ranging from 8 to 12 days (Gubler, 1992). High ambient temperatures speed up virus replication and shorten the EIP, whereas cooler temperatures prolong the EIP (Watts and others, 1987). Thus, the time interval between cases ranges from 10 to 30 days. A single dengue case can infect many mosquitoes. Since daily survival rate of *Ae. aegypti* has been estimated between 66 and 86 percent (Sheppard and others, 1969), only a few mosquitoes survive the extrinsic incubation period of the virus. In a recent series of mark-release recapture studies carried out in Florida, Puerto Rico, the daily probability of survival has been estimated to be between 55 and 85 percent (Tom Scott, University of California, Davis, oral commun., 1995; Edman and others, in press). On average, given a daily survival rate of 76 percent, only 1 of 10 mosquitoes feeding on a viremic person would survive more than 8 days.

Dengue is a seasonal disease (Gubler, 1992; Centers for Disease Control and Prevention, 1993). In Puerto Rico, transmission generally increases in July or August and extends through January (Centers for Disease Control and Prevention, 1993). The underlying factors inducing this seasonality are poorly understood. High vector population densities or excess rainfall (which increase the number of vector breeding sites) (Scanlon, 1966; Monath, 1985; Watts and others, 1987) have been implicated, but attempts to correlate adult *Ae. aegypti* abundance and survival to dengue incidence have not been conclusive (Sheppard and others, 1969; Tonn and others, 1969; Yasuno and Pant, 1970; Pant and Yasuno, 1973). Ambient temperature may be the most important factor (Burke and others, 1980; Watts and others, 1987). In contrast, a study specific to Puerto Rico found that *Ae. aegypti* populations increased about 6 to 8 weeks prior to the annual appearance of dengue cases, and that the onset of the rainy season preceded *Ae. aegypti* increases by an additional 2 to 3 weeks (Moore and others, 1978). Moore (1985) used multiple regression models to predict Breteau indices (number of *Ae. aegypti* positive containers per 100 houses sampled) from rainfall data. Rainfall appeared to be an important constraint on the south coast (Ponce, Guayama), but

not in the north or western part of the island (Arecibo, Mayagüez). Furthermore, temperature was not a useful predictor of larval abundance in these studies.

The most important factor influencing *Ae. aegypti* abundance is the presence of appropriate larval habitats. In Puerto Rico, a variety of artificial and natural containers, including 55-gallon drums, discarded appliances, used tires, buckets, small plastic containers, flower-pot bases, and, less frequently, bromeliads serve as habitats for immature *Ae. aegypti* (Moore and others, 1978; Moore 1983, 1985; Gubler, 1992). The presence of larval habitats varies dramatically within an area and is controlled entirely by individual members of the community. In theory, households that eliminate mosquito breeding sites would not be at risk of dengue infection, assuming the flight range of *Ae. aegypti* is short (50 m). However, there is evidence that *Ae. aegypti* may move substantial distances (km) and thus contribute significantly to the rapid dispersal of dengue viruses (Sheppard and others, 1969; Bond and others, 1970; Hausermann and others, 1971; McDonald, 1977; Trpis and Hausermann, 1986; Reiter and others, 1995). Molecular studies on field-collected eggs in Puerto Rico indicate that *Ae. aegypti* oviposit at a large number of sites (Apostol and others, 1994, 1996). In addition, *Ae. aegypti* commonly takes blood several times during a single gonotrophic (egg laying) cycle (MacDonald, 1956; Gould and others, 1970; Scott and others, 1993a,b). Thus, a single infected mosquito could infect an entire household within a few days. The energy needed to support dispersal and the observation that in urban areas many *Ae. aegypti* do not feed on sugar (Van Handel and others, 1994) indicate that multiple feeding behavior and the dispersal of *Ae. aegypti* could be important determinants of dengue virus dispersal.

Studies of Vector-Borne Disease Using GIS

GIS is especially appropriate for a landscape epidemiological approach to the study of disease that attempts to identify environmental factors that determine the temporal and spatial distribution of both vectors and disease (Beck and others, 1994; Pavolosky, 1966). The spatial distribution of some diseases results from direct exposure to an environmental factor such as a contaminated water source or air pollution from a factory. The effect of the environment is often indirect. For example, temperature, rainfall, and humidity influence the presence, development, activity and longevity of *Ae. aegypti*, as well as the development of the dengue virus within the mosquito vector. This landscape epidemiological approach ultimately attempts to predict areas with highest risk of disease transmission.

GIS technology has been successfully applied to the studies of the vectors of numerous water related diseases, including the mosquito vectors of Rift Valley fever, Saint Louis encephalitis, and malaria (Beck and others, 1994; Lithicum and others, 1987; Wood and others, 1991, 1992), and to the snail vector of the disease schistosomiasis (Cross and others, 1984). In each of these examples, however, the aquatic habitats studied were large; remotely sensed data was used to identify larval habitats such as temporary or permanent ground pools, marshes, rice fields, rivers or streams. This approach is not applicable to dengue since the larval habitats of *Ae. aegypti* are small containers associated with human habitations.

The rationale for spatial studies of dengue case patterns is that the spatial dependence may significantly affect interpretation of dengue surveillance data because parameters and processes important at one scale are frequently not important or predictive at another scale (Liebhold and others, 1993). Biased or spurious results due to disregard for spatially dependent variables have been reported for ecological (Liebhold and others, 1991; Rastetter and others, 1992), landscape (Meentemeyer and Box, 1987; Turner and others, 1989), and epidemiological (Lecoustre and others, 1989; Morrison and others, in press) studies. There are a variety of spatial statistical approaches including geo-statistics (for example, Kriging, reviewed by Cressie, 1993), spatial autoregressive modeling (Cliff and Ord, 1981), and pattern analysis (Cliff and Ord, 1981).

The principal advantages of a GIS are its spatial analysis capabilities (Clarke and others, 1996). These include data visualization and exploratory data analysis which allow investigators to interpret spatial data. The graphics and animation features embedded within a GIS are highly effective in demonstrating the

spread and dispersal of disease over time. Moreover, spatially-referenced data facilitates the use of spatial statistical procedures that are common in the geography, geology, and statistics literature but have not been widely applied to epidemiology. In general, spatial statistical methods account for spatial dependence of data (Cressie, 1993). In contrast, most ordinary statistics assume that observations are independent. Disease incidence rates commonly exhibit spatial autocorrelation. Autocorrelation refers to the observation that samples collected close to one another are often more similar to one another than they are to samples collected further away, whether in space or time (Robertson, 1987). The spatial statistical methods utilized in the project are based on theory of spatial point processes, also called pattern analyses (see Cliff and Ord, 1981; Bailey and Gatrell, 1995), that consider the distance between each point and all other points to describe and analyze point patterns (Boots and Getis, 1988; Cressie, 1993; Gatrell and others, 1996; Gatrell and Bailey, 1996) and characterize disease clustering in time and space (Knox, 1964; Barton and others, 1965; Mantel, 1967; Marshall, 1991). For dengue, these methods can be applied to identify areas of increased transmission ("hot spots"), dispersal and clustering patterns, and to make spatial comparisons between cases with different demographic characteristics.

The most common way to detect spatial structure (departure from complete spatial randomness) in point pattern (for example, dengue case locations) is to estimate a K-function. Informally, this measures the extent to which the local intensity (density) of points (events) in one small region (for example, a house, block, neighborhood) is correlated with that in an adjacent small region. Formally, it is defined as the expected number of point events within a fixed distance of an arbitrarily chosen event, scaled by the overall density of point events (Gatrell and Bailey, 1996). Gatrell and Bailey (1996) reanalyzed case control data of childhood cancer rates in South Lancashire, England, and incidence rates of Burket's lymphoma and Ebstein-Barr virus (EBV) in Uganda. They showed that the clustering patterns of cancer patients and healthy controls were the same, and found some evidence of space-time clusters of Burket's lymphoma and EBV-virus. Local statistical (Getis, 1996) methods have been employed to locate hot spots of human immunodeficiency virus (HIV) transmission (Ord and Getis, 1995) and more recently demonstrated that LaCrosse encephalitis cases in Illinois clustered within 3.0 km of the city of Peoria and that transmission was concentrated around hardwood ravines and tire piles (Kitron and others, 1997).

Because disease surveillance is conducted to describe who becomes infected, where, and when, an understanding of the spatial and temporal patterns of dengue will identify at what geographic scale cases occur, and thus determine the scale at which control measures should be applied. From these spatial patterns, hypotheses will be generated on the possible mechanism of dengue virus movement within a community, whether by human movement, mosquito dispersal or both. In addition, pattern differences among distinct demographic risk groups may reveal important differences among these groups.

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GEOGRAPHIC SETTING

The main island of Puerto Rico is 179 km long by 58 km wide (8,875 km^2) with a population of slightly more than 3.5 million (fig. 1). Puerto Rico consists of a mountainous core encircled by an elevated coastal plain. Two mountain ranges, the Sierra de Luquillo and the Cordillera Central, cross the island from east to west (fig. 1). The north central and northwestern regions of Puerto Rico have undergone karstification —over a period of millions of years; rainfall and ground water have seeped through the primary structural lines and joints of the porous limestone terrain and formed extensive caves, sinkholes, and left large remnant limestone hills (mogotes). Puerto Rico is composed of 78 municipalities ranging in population from 1,542 for the Isla de Culebra to 437,745 for San Juan. The area of each municipality ranges from 12.5 km^2 (Cataño) to 327.2 km^2 (Arecibo). Annual rainfall in Puerto Rico ranges from 76 cm (29.9 in.) to more than 500 cm (197 in.) and varies across the island (fig. 2a). Mean annual temperature varies with elevation, from 67.0 degrees Fahrenheit (°F) at the highest peaks (1,000 to 1,300 m) to 89.2 °F along the coastal plain (fig. 2b).

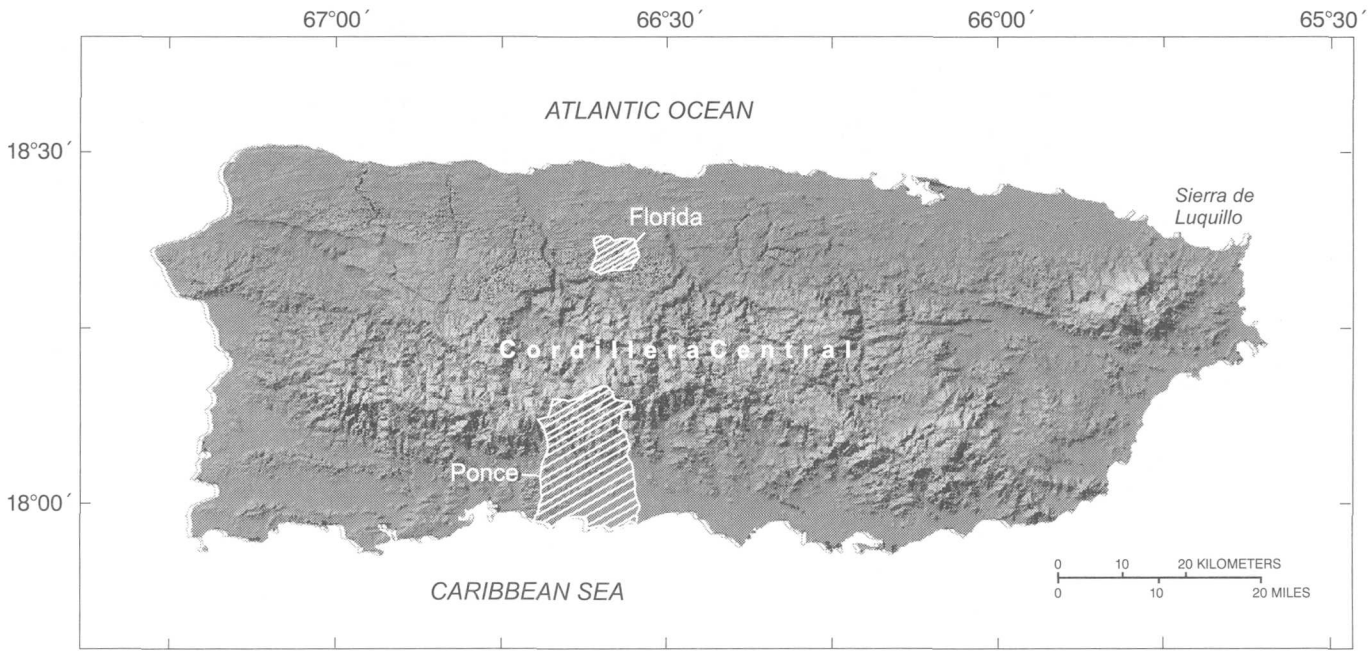

Figure 1. Location of the municipalities of Florida and Ponce, Puerto Rico.

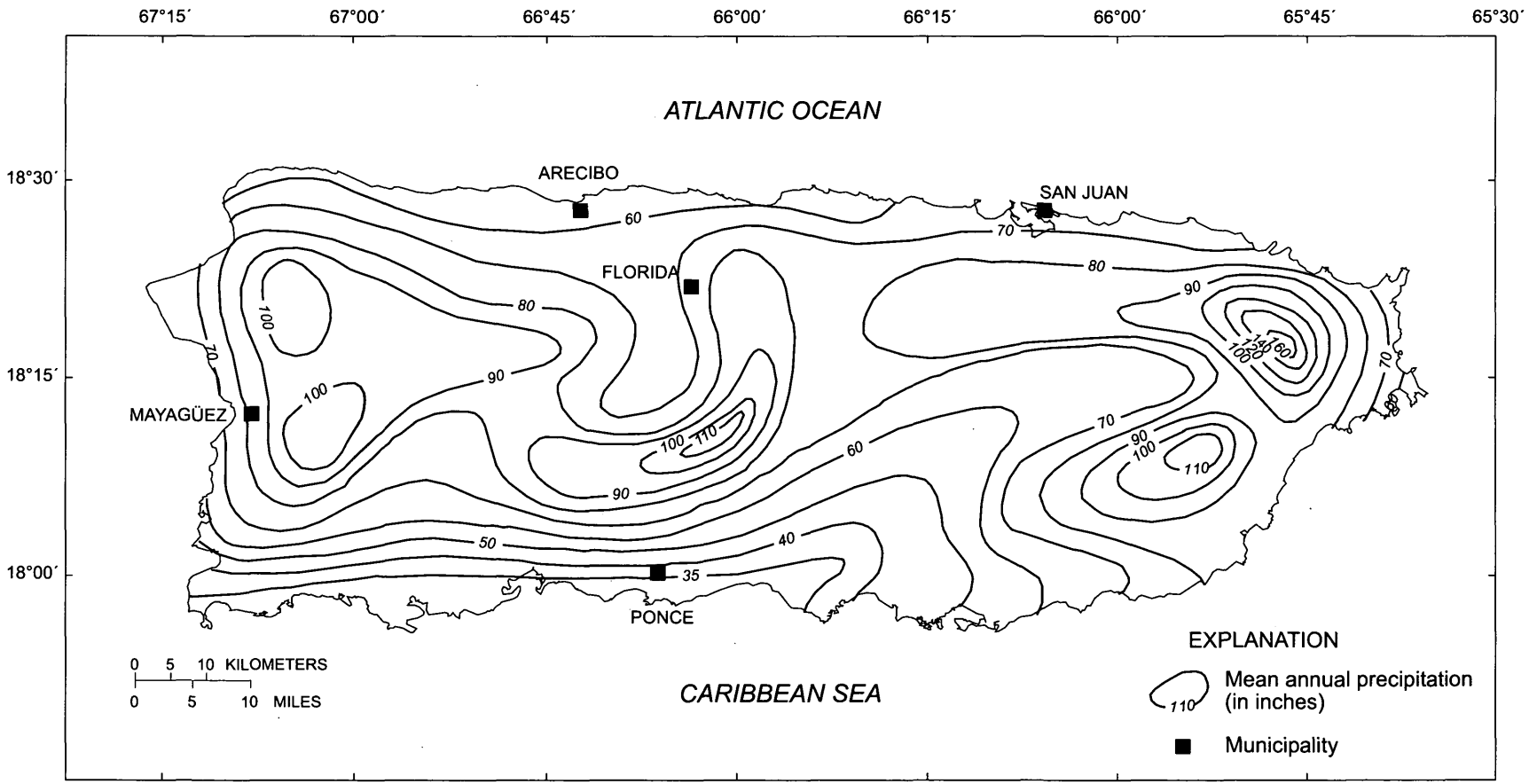

Figure 2a. Mean annual precipitation in Puerto Rico (Data from Calvesbert, 1970).

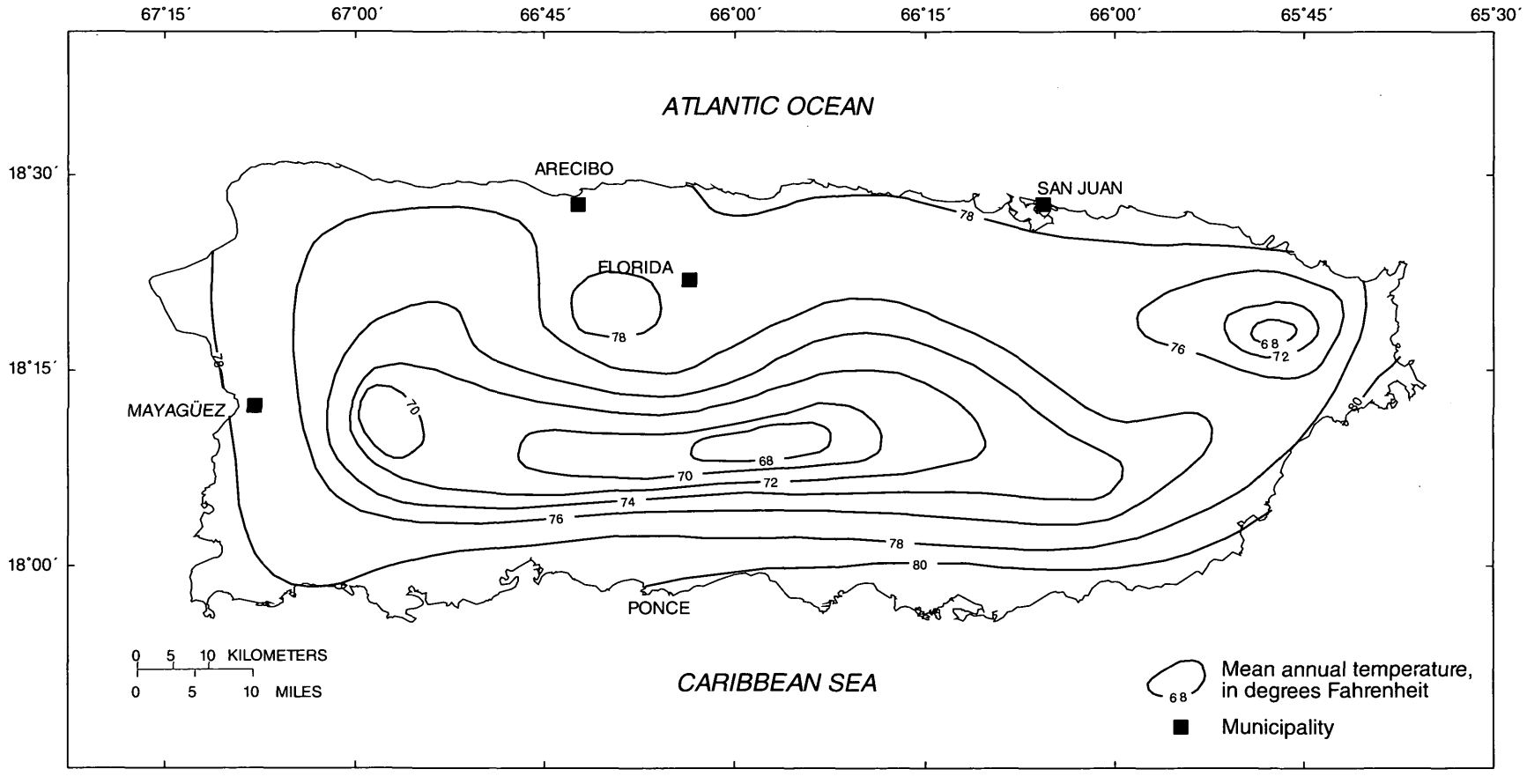

Figure 2b. Mean annual temperature in Puerto Rico (Data from Calvesbert, 1970).

STUDY AREAS

The municipality of Florida (26 km^2) is a small suburban community of 8,689 people (U.S. Census Bureau, 1990) located in the hills of north-central Puerto Rico (fig. 1). Florida had the highest incidence rate for dengue during 1991 (15.7 cases per 1,000 people) (Rodríguez-Figueroa and others, 1995); virus isolations indicated that dengue-2 was the predominant serotype circulating in the area (27 of 29 samples tested, 93.1 percent). The municipality consists of 9 well-defined "urbanizations" (neighborhoods or housing developments), 2 public housing projects, and 7 rural areas. Several neighborhoods (at an average elevation of 200 m above sea level) are separated by mogotes, steep, uninhabited limestone hills. Although, these features do not prevent movement of humans, they presumably act as a natural barrier for *Ae. aegypti.*

The Florida community was originally selected because it was used for a serological and an entomological survey conducted by CDC during a dengue outbreak in 1991 (Centers for Disease Control and Prevention, unpublished data; Rodríguez-Figueroa and others, 1995). Accordingly, dengue cases reported to CDC during 1991 and 1992 from Florida were used for this study. Community awareness of the disease was found to be high due to the large number of cases and the occurrence of four cases of DHF (one fatal) (Rodríguez-Figueroa and others, 1995). U.S. Census Bureau (1991) data indicate Florida is a rather homogenous lower-middle class community with a readily accessible government health center (Centro de Diagnóstico y Tratamiento, CDT).

The municipality of Ponce (300.7 km^2) (fig. 1) includes the city of Ponce, one of Puerto Rico's larger cities (fig. 1). Most of the population (187,749) is concentrated in the city and adjoining suburbs. Ponce has approximately 90 urbanizations, 20 housing projects, and 30 rural communities. The municipal boundaries extend from the coast on the Caribbean Sea to the Cordillera Central. This report includes data from the 1994-95 dengue season, one of the most severe ever observed in Puerto Rico (over 20,000 cases). In Ponce, however, transmission remained at endemic levels during the 1994-95 dengue season. Ponce was chosen as the second study area because it contrasts with Florida in size, population, geography, climate, and dengue transmission dynamics (endemic versus epidemic transmission). In addition, the quality of the address data and zoning maps for Ponce was higher than for other areas of Puerto Rico.

METHODS OF INVESTIGATION

Figure 3 is a flow chart that links the data sources and analyses to objectives of the project. Briefly, the dengue case data used for this study were obtained from a laboratory-based surveillance system of the San Juan Laboratories, Dengue Branch, Division of Vector Borne Infectious Disease, National Center for Infectious Diseases, Centers for Disease Control and Prevention (Gubler and Casta-Vélez, 1991). For microgeographic studies, the residential address of each reported dengue case was georeferenced by finding its location on a base map. Base maps containing data on roads, hydrography, topography, lot size and location from Florida and Ponce, and municipality boundaries for Puerto Rico, were collected, processed, and digitized for use with GIS. Population data, including demographic information and housing characteristics, were obtained from the 1990 U.S. Census Bureau topologically integrated geographic encoding and referencing (TIGER) files (U.S. Census Bureau, 1991). A vector-based GIS software (ARC/INFO, Environmental Systems Research Institute, Redlands, CA.) was used to obtain the geographic coordinates of residential addresses of patients, produce maps of weekly dengue incidence, and calculate distances between case pairs for use in nearest case pair and spatial statistical analyses. For macrogeographic analyses, dengue case data were extracted from the CDC surveillance data base and summarized by municipality. These data were linked to municipality boundary coverages (a digital version of a map forming the basic unit of vector data storage in ARC/INFO) to produce monthly serotype maps and to develop municipality based climate zones. Accordingly, the following discussions are presented: (1) the characteristics and limitations of the dengue surveillance database, (2) development of digital zoning maps and population databases, (3) how dengue cases were georeferenced and weekly maps were produced, (4) characterization of microgeographic dengue case patterns, and finally, (5) the methods used for the macrogeographic analyses.

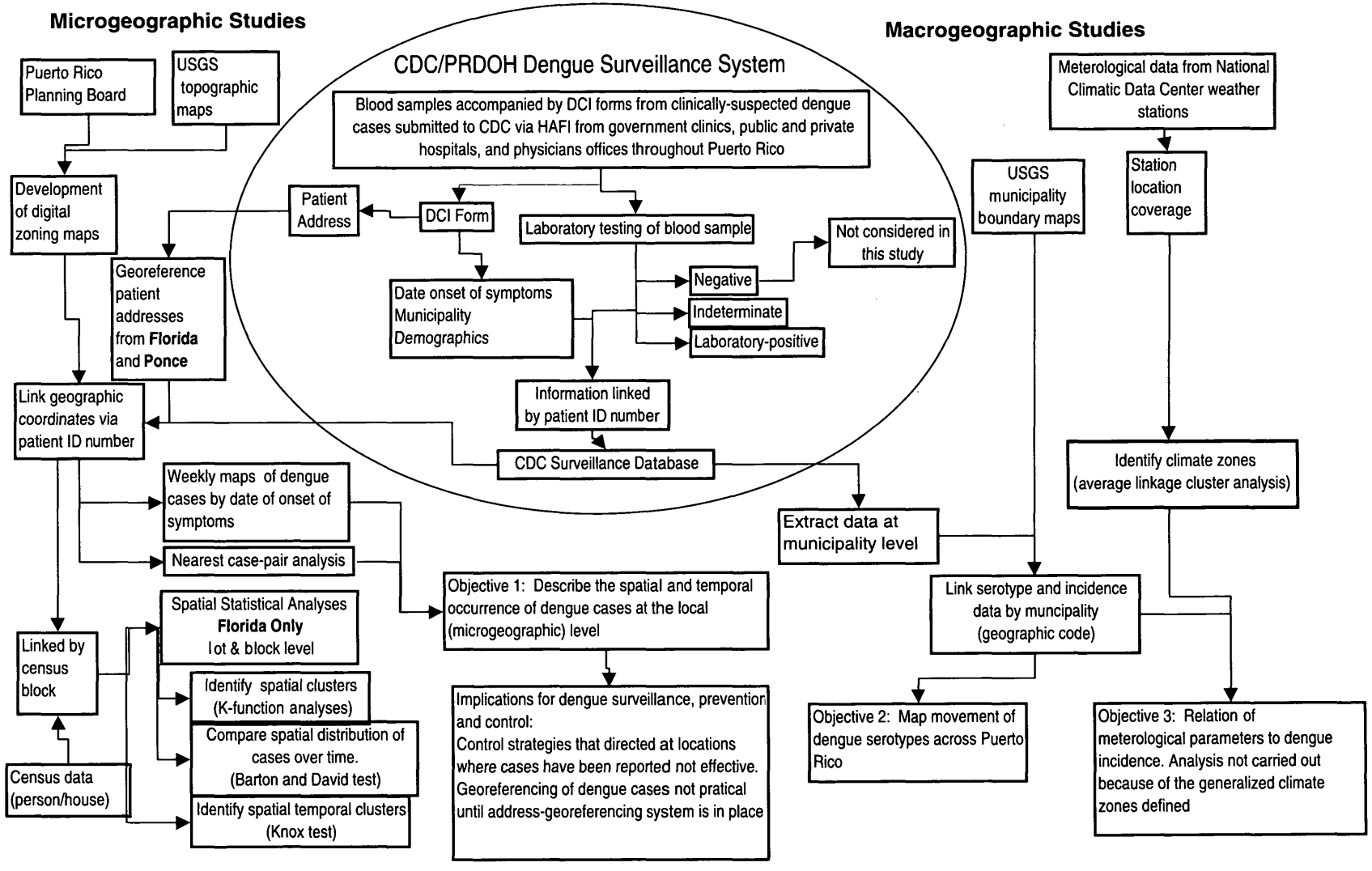

Figure 3. Relation of data sources and analysis to the objectives of the project.

Characteristics and Limitations of Dengue Surveillance Data Base

Blood samples from clinically-suspected dengue cases are submitted to CDC via HAFI from government clinics, public and private hospitals, and physician's offices throughout Puerto Rico, along with a standardized information form (dengue case investigation, DCI) for each sample. The DCI form lists information on the residential address, age, sex, date of onset of symptoms of the patients, and a checklist to indicate the symptoms and signs reported by the patient or elicited during the evaluation. Serum specimens are tested for anti-dengue IgM by the IgM antibody-capture enzyme-linked immunosorbent assay (ELISA) method (MAC-ELISA) (Burke and others, 1982; Kuno and others, 1987; Gubler and Sather, 1990). Specimens with positive virus isolation or borderline results by MAC-ELISA are assayed by hemagglutination-inhibition (HI) testing (adapted to microtiter) (Clark and Casals, 1958) or IgG-ELISA (after October 1991) (Chungue and others, 1989). Serum specimens collected less than 6 days from the onset of illness are applied to C6/36 mosquito cell cultures for virus isolation (Gubler and others, 1984; Kuno and others, 1985). Dengue viruses are identified using serotype-specific monoclonal antibodies in an indirect fluorescent antibody test on virus-infected cell cultures (Gubler and others, 1984).

Dengue cases were classified as confirmed, probable, indeterminate, or negative (Rigau-Pérez and others, 1994). In our study, probable (clinically compatible illness with a positive IgM result or very high IgG titer [>1:163,840, ELISA; > 1:1,280, HI]) and confirmed (dengue virus isolated from patient serum or a fourfold or greater change in anti-dengue antibody titer in paired serum samples) were considered together as laboratory-diagnosed or laboratory-positive cases. Single specimens negative for virus and for IgM were considered indeterminate, if collected 5 or less days from the onset of illness. The absence of IgM was considered a negative dengue diagnosis if specimens collected at 6 or more days after the onset of symptoms and were not considered in this study.

Laboratory, clinical, and demographic information, and the patient's municipality of residence (assigned a geographic code) were entered into a Foxpro database. The geographic codes were used for weather analyses as well as to extract all of the cases reported in the Florida and Ponce municipalities. An address database was created from the information sheets that accompanied the blood specimens that could be linked to the surveillance database by a unique identification number assigned to all the cases reported by the CDC.

Certain limitations arise when using data from a surveillance system, including under-reporting, **reporting bias**, **recall bias**, and technological limitations. The clinical spectrum of dengue includes a large percent of asymptomatic or mild cases that will not be detected by the surveillance system. Asymptomatic dengue in Florida was about 53 percent (Rodríguez-Figueroa and others, 1995). Our analysis assumed that the dengue cases reported to the CDC surveillance system represented a random (unbiased) spatial sample of all infected individuals. This assumption is reasonable if the cases captured by the system are unbiased (the sample represents most of symptomatic dengue cases occurring in the area) and the characteristics (demographics and spatial distribution) of undetected cases (asymptomatic) do not differ significantly from detected cases (symptomatic). Several characteristics of the 1991-92 Florida outbreak support these assumptions:

(1) Awareness of dengue was high in Florida, probably because of the high incidence of the disease and the government presence (vector control and media campaigns);

(2) Florida is a small tight-knit community served by a readily accessible government health clinic, and the probability that dengue cases would be treated at this center or regional hospitals that participate in the CDC surveillance system was high; and

(3) The demographic characteristics of the community were relatively homogeneous, reducing the potential for demographic risk factors in **confounding** the spatial pattern of observed cases.

Another type of problem is recall (memory) bias. The date of onset of symptoms used in this study is reported by the patients themselves; although the time interval between exhibiting symptoms and reporting to a physician is short, some degree of error must be expected. Another limitation is mapping the residential address data of the patient. The travel history (both foreign and domestic), schools attended, and workplace of a case are all important variables that were not available. Furthermore, the quality of the address data was variable. In many cases the data were incomplete. There were also cases when the reported address was that of a relative or friend living in the area. Because of these problems, numerous field checks of addresses were necessary.

These limitations suggest that the spatial analyses presented later in the report are exploratory in nature and generate hypotheses rather than confirm them. Whereas it may not have been appropriate to extrapolate conclusions beyond "reported" cases, these patterns may prove to be very significant because they are derived from a conservative sample of all dengue cases.

Finally, technological and logistic limitations of the virological and serological techniques used to detect dengue cases are relatively insensitive, and may result in a high percentage of "indeterminate" cases. Because the positivity rate (number of laboratory-positive cases divided by laboratory-positive plus negative dengue diagnoses) was greater than 80 percent in Florida (Centers for Disease Control and Prevention, unpublished data), at least this proportion of the indeterminate specimens was judged to be true dengue cases; therefore, these cases were included in the spatial pattern analysis. In contrast, for Ponce the positivity rate was less than 20 percent (Centers for Disease Control and Prevention, unpublished data). For the case of Florida, however, some of the indeterminate cases probably were not dengue, and therefore, all spatial pattern analyses described in this study were carried out for two groups of cases: a combination of laboratory-positive cases and indeterminate cases (hereafter called *all cases*), and laboratory-positive cases.

Development of Digital Zoning Maps and Population Data Bases

Road, hydrologic, and topographic data were obtained from USGS Digital Line Graph (DLG) files of topographic quadrangles (1:20,000) (U.S. Geological Survey, 1986). The Florida coverages were extracted from the Florida (1957) and Barceloneta (1969, photorevised 1982) quadrangles, whereas the Ponce coverages were derived from the Ponce (1970), Playa de Ponce (1970), Peñuelas (1972, photorevised 1982), Jayuya (1960, photorevised 1982), and the Adjuntas (1960) quadrangles. The position of individual housing lots was obtained from digital zoning maps (1:2,000) prepared by the Puerto Rico Planning Board from existing zoning maps of the area. The original zoning maps were developed from aerial photographs with revisions based on field checks. Four zoning maps (Florida tiles 6[1980], 10[1988], 11[1980], and 14[1984]) were available, but they only included a portion of the populated areas in Florida and did not show all individual lots in the downtown area. All lots were counted in the field, and the line work was added to show their positions. In urbanized areas, lot sizes were measured with a measuring wheel; in less developed regions, the number of houses was counted between two intersections and the lot locations were estimated. This formed a polygon coverage (Florida lot coverage), containing 2,989 lots, that was used for statistical analyses (fig. 4).

Digital zoning maps covering urban regions of Ponce were obtained from the *Oficina de Ordenación Territorial de Ponce*. The digital files for approximately 120 zoning maps were converted from a MapGrafix (Comgrafix, Inc., Clearwater, Florida) to ARC/INFO format. The original maps were developed from aerial photographs at an approximate scale of 1:12,000 that were suitable for preparation of 1:2,000 scale planimetric mapping by stereo compilation methods on a first order plotter by the Caribbean Aerial Survey. In addition, a commercially available map guide of Ponce (Metrodata, 1994) developed from the same line work, but containing lot numbers (addresses), was used to locate specific addresses.

In order to estimate the number of people living in each lot observed on the Florida zoning maps described above, TIGER census block boundaries (U.S. Census Bureau, 1991) were digitized for use in the GIS. Each lot observed on a zoning map was assigned to the appropriate TIGER census block, based on house counts made in the field and on physical boundaries defined in the files (fig. 4). Basic information on demographics, including number of people by sex and age group and housing (number of occupied and vacant housing units) was linked to the TIGER census block coverages by a unique census tract and block number. Population estimates based on TIGER data must be interpreted with caution for the following reasons:

(1) The population data used in this study were collected in 1990 and were applied to the 1991-92 transmission season;

(2) The locations of vacant houses were unknown, decreasing the population estimate for areas with many vacant houses;

(3) Multi-family dwellings could not be identified, possibly inflating the person-per-household estimates for certain blocks; and

(4) A single population estimate was assigned to all of the housing units within an individual TIGER census block, masking variation within the block.

Georeferencing Dengue Cases and Production of Weekly Maps

To create a point coverage containing the geographic location of each dengue case reported in Florida and Ponce, the address of each case was obtained from the DCI form and subsequently located in the field and recorded on a base map (paper copy). If the address on the DCI form was incomplete, absent or incorrect (a number or location that did not exist) an effort was made to locate the patient's residence. Local health workers, community leaders, and local store owners were heavily relied upon to find the "missing" addresses. In most cases, the patient's address was represented by the centroid of a lot polygon on the appropriate base map. When the patient's residence was not represented by a single lot (polygon), the position of the center of the house was measured directly from a known intersection on a zoning map or USGS topographic map, by using a measuring wheel. For a small number of cases located in areas where there were no physical reference points to locate the case address, a Geopositioning System (GPS) was used to determine the geographic coordinates of the case's houses.

The geographic positions of each case were converted into an ARC/INFO coverage using an electronic digitizing tablet or by digitizing the position directly on a base map located on the computer screen. Each case location was linked to the case information by a unique CDC case identification number.

A computer program was written in Arc Macro Language (AML) to create a series of maps showing the position of each reported dengue case for each week during a transmission season. The cases were plotted according to their reported date of onset of symptoms, using unique symbols for the dengue case definition (laboratory-positive and indeterminate).

Characterization of Dengue Case Patterns

A simple method for studying the spatial-temporal distribution of dengue cases was developed for this study and is referred to as "nearest case pair analysis." A data matrix containing the distance and time interval between every possible case pair combination was generated using the ARC/INFO point distance commands and was imported into S-Plus (Statistical Sciences, 1993), a statistical software package. Also included in this matrix was the State Plane coordinates and date of onset of symptoms for each member of the case pair. Various subsets of this data matrix could be extracted in order to study the distribution of case pairs in both space and time. Based on our knowledge of the incubation (extrinsic plus intrinsic) periods for dengue, case pairs separated by more than 30 days were removed from the data matrix, since it was highly unlikely that a case occurring more than 30 days earlier could be the source of infection of the second occurrence. Assuming cases that are closer together geographically are more likely to be related, the case pair with the shortest distance interval for each case was extracted to make a new data matrix containing the "nearest case pairs." These matrices were used to generate histograms displaying the frequency distributions of nearest case pairs in time and space.

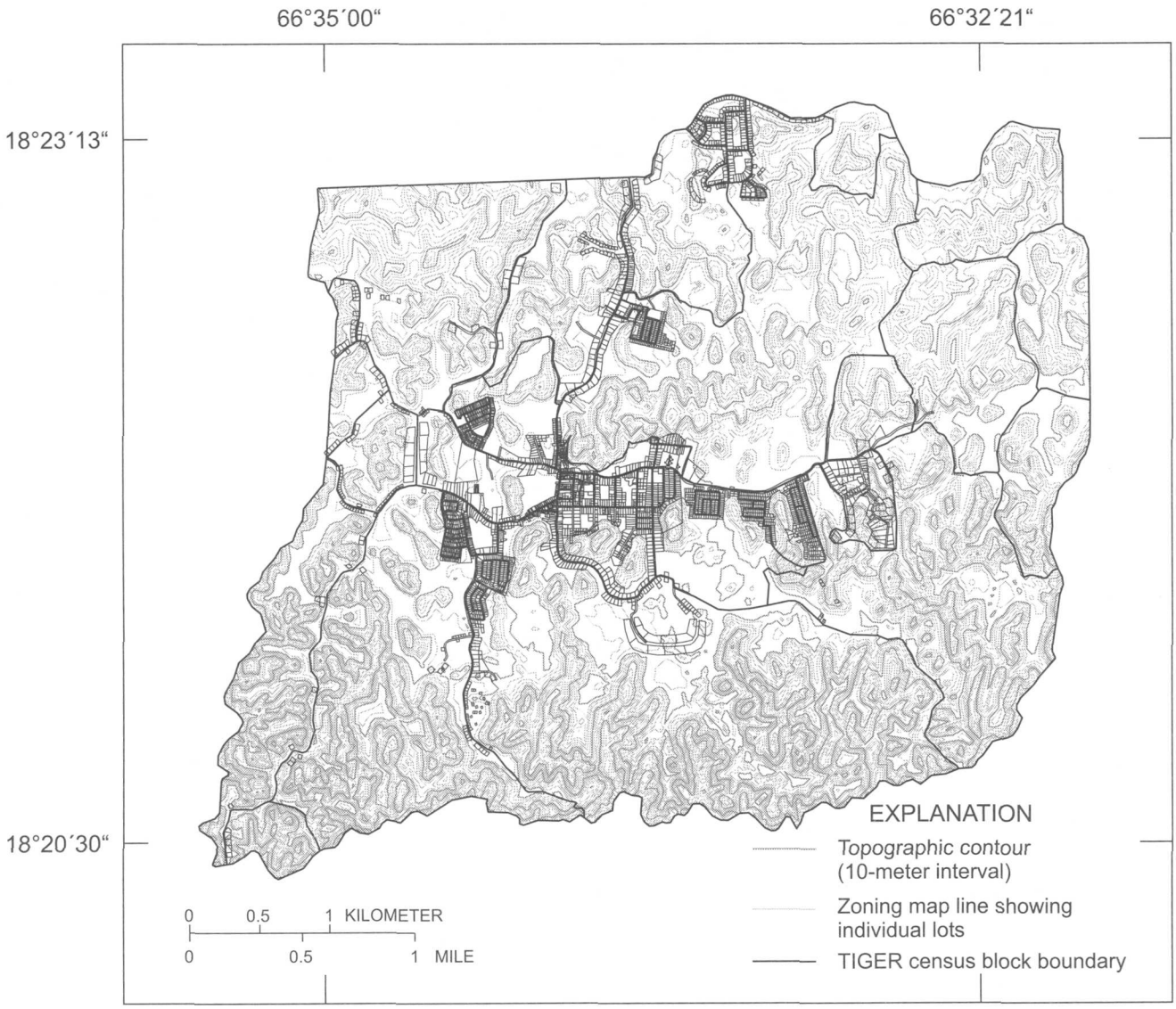

Figure 4. Census blocks (1990) in Florida, Puerto Rico.

Because of the higher quality of the dengue case data from the municipality of Florida epidemic, these data were analyzed by a series of exploratory spatial analyses. These analyses were conducted at two levels of spatial resolution: individual houses (lot level) and census block level (groups of houses taken together). The surveillance data give the location of the lots which are the residential locations of reported dengue patients during the 1991-92 transmission season. The more general level of resolution is the census block. Reported cases were grouped into the 83 blocks (fig. 4) that comprise Florida and compared with demographic information (age, sex, and population density).

Analysis at the Lot Level

To identify spatial clusters of reported dengue cases, the K-function for the pattern of clinically reported cases was calculated and contrasted with the K-function for the population of Florida represented by houses (lots). The K-function (Ripley, 1976, 1981; Diggle, 1983; Getis, 1984; Gatrell and others, 1996) was calculated from the distances between all pairs of points at a series of distances, d. K(d) values are cumulative. For this study, K(d) was calculated at 5-m intervals using a modification of Ripley's original K-function (Besag, 1977). The equation for the modified K-function is

$$K(d) = \sqrt{A\Sigma_i\Sigma_j w_{ij} I_d / \pi n (n-1)}$$

where I_d is 1 if the distance between points is less than or equal to a distance d, w_{ij} is a weight (greater than 1) that takes into account a boundary condition when the distance to the boundary of the study area from one point in a pair of points is shorter than the distance between the pair members, A is the area of the study region, and n is the total number of points. The K-function is represented by a plot of d (x axis) by K(d) (y axis). The K-function for the location of all lots containing a house was calculated where each lot was weighted by the estimated number of residents (Getis, 1984). Since the population estimates for each lot were based on census data, the assumption was made that all members of the Florida community were susceptible to dengue. Although some members of the community were immune to dengue-2 virus at the time of the epidemic, the absence of previous dengue activity (lowest municipal incidence rates in Puerto Rico during the previous 5 years) allowed us to assume that this number was low. Clusters were then identified by comparing the slopes of the K-function and the relative magnitudes of the K(d) values between the case and population functions. For example, if the K-functions of single and high-rise buildings are compared, the K-function of the high-rise buildings, clustered in commercial areas, would be steeper than that of the more dispersed single level dwellings. In addition, this approach was extended to determine whether differences in the spatial pattern of the disease exist by age, sex, or laboratory diagnosis.

The Barton and David (Barton and others, 1965) test was used to determine whether spatial patterns of dengue cases varied by temporal cluster. A temporal cluster includes the successive cases that are separated by less than the average time interval between all successive cases. The spatial coordinate centroids of each temporal cluster were identified and compared. The main value of this approach is that it can detect directional changes, that is, movement over time from one neighborhood to another. The null hypothesis of this test is that there is no association between the coordinate centroids from time period to time period; this indicates that there is no major change in the spatial pattern of cases. The test cannot distinguish between a temporal disease cluster that is clumped around a central point from another cluster that is widely dispersed around the same point, nor is it affected by size (number of cases) differences in the temporal clusters. This test was used to identify changes in pattern overall, and by sex and age. For age, two different group types were studied: greater than or equal than 15 years and less than 15 years of age.

The Knox (1964) test was used to identify time-space clusters of cases. This method tests for possible interaction between the distance and time separating cases, that is, whether the number of case pairs occurring in a particular time-space window (for example, a case pair separated by 25 m and 4 days) is significantly different from the number of cases expected in the same window given the total number

of cases, considering the period of time over which the epidemic took place, and the extent of the spatial distribution of cases. Only pre-planned comparisons, based on specific hypothesis should be tested. For this study, the critical time periods tested were based on the gonotrophic cycle length of *Ae. aegypti* (3-5 days) and the spatial clustering patterns identified by K-function analysis.

To identify whether local "hot spots" of dengue cases exist within the broad pattern of cases, a local K-function analysis was performed (Getis, 1984). Here the focus is on each individual case, one at a time. A search was made of the local population distribution to identify significantly more cases within a specified distance from each case than would be expected by chance. If a series of nearby cases have in their immediate neighborhood significant numbers of nearby cases, that group of cases can be considered a spatial cluster. That is, a K-function is calculated for each individual house, and will identify specific clusters of cases (for example, on one city block), rather than describe a municipality-wide clustering pattern (for example, dengue cases tend to cluster at the level of a city block throughout Florida).

Analysis at the Block Level

In the 1990 census, Florida was divided into 83 blocks for which a great deal of demographic and socioeconomic data were collected. For each block (fig. 4), the location of the population center was estimated. These locations served as the basis for comparing the number of cases per block during the transmission season with population, age, sex, and population household density. No income data or usable surrogates for income were available for Florida. Data on house values and non-owner house rent were available, but these were difficult to evaluate because home owners, especially of high value houses, were dispersed widely among census blocks which otherwise would be considered as low income areas. Thus, persons per household were used as a rough surrogate for socioeconomic status. Again, K-function analysis was used to identify any larger scale clustering patterns. K(d) values were calculated up to a distance of 1,000 m from the center of each block to compare the pattern of cases versus the population in each census block. In this way, it could be determined whether blocks had more cases than expected, given the size of the population blocks.

Characterization of Dengue Incidence Pattern for Puerto Rico

Macrogeographic studies, using dengue incidence rates for municipalities, were used to study serotype movement throughout Puerto Rico and to relate rainfall and temperature patterns to seasonal changes in dengue incidence. The number of individual dengue serotype isolations was displayed for each municipality by month, for the period between June 1988 and May 1994 using ARC/INFO software.

Daily temperature and rainfall data from 85 meteorological stations in Puerto Rico were obtained from National Climatic Data Center tape files, of these, 34 stations had complete data for the period between January 1988 and March 1994. An average linkage clustering procedure (Kalkstein and others, 1987) was performed on monthly mean precipitation and maximum and minimum temperatures for the 34 stations to classify climatic regions for Puerto Rico. Because dengue incidence data for the island of Puerto Rico are only available by municipality, the climate zones defined had to maintain municipality boundaries. However, landscape and climate within many of the municipalities vary greatly; for example, the municipality of Ponce extends from the coastal plain to the Central Mountain Range. Consequently, only three generalized climatic zones could be defined. These zones were not consistent with previous climatic classifications of the island, irrespective of municipality boundaries (see figs. 2a, b; Calvesbert, 1970; Holdridge, 1967). Defining climate zones based on more appropriate parameters (not limited by municipality boundaries), and then georeferencing dengue cases at a finer spatial scale were beyond the scope of the present study. Approximately 3.4 person-hours of labor was necessary to georeference each dengue case in the microgeographic studies; applying this estimate to an average of 15,000 dengue cases per year for all of Puerto Rico, would have required approximately 255,000 person-hours to georeference all of the

reported dengue cases during the 5-year study period. Although, additional weather analyses using these climate zones would be inappropriate, this attempt to use data collected on different geographic scales illustrates an important limitation of GIS. Disease surveillance data are typically summarized for politically rather than environmentally defined areas and until researchers are able to georeference cases at a finer scale, GIS alone will not make these types of spatial analyses possible.

CHARACTERIZATION OF THE GEOGRAPHIC SPREAD OF DENGUE IN THE MUNICIPALITIES OF FLORIDA AND PONCE

Locating the residential address of each reported dengue case required multiple visits to both Florida and Ponce. As mentioned previously, many of the addresses reported to CDC were incomplete or difficult to locate in the field. It was estimated that address location alone required between 0.75 and 1 person-hour per case. If computer time, including data entry, digitization, and weekly map preparation are included, the labor required to map each dengue case was estimated at 3.4 person-hours. The numbers of georeferenced cases are summarized in table 1. The basic demographic characteristics of the cases in each municipality are compared in table 2.

The most notable difference between the two municipalities was found in the percentage of indeterminate laboratory results; 76.8 percent in Ponce compared to 52.4 percent in Florida (χ^2=50.2, df=1, P <0.0001). This difference is probably attributable to circumstances related to the 1994 dengue epidemic, the worst island-wide dengue epidemic in 25 years. The CDC laboratory was unable to process all of the blood samples it received and many samples had to be frozen and stored. After Ponce was selected as a study area, the samples from this municipality were tested for anti-dengue IgM but not processed for the virus.

The weekly reported dengue cases for Florida between June 21, 1991, and January 4, 1992, when 94.2 percent of the reported cases occurred, are shown in figure 5a. The reported cases in Ponce for the entire June 1994 to May 1995 transmission season are shown in figure 5b. Weekly maps of dengue cases by their date of onset of symptoms were produced at 1:2,000 and 1:20,000 scales for Florida (figs. 6a-6d) and Ponce (figs. 7a-7e), respectively. These scale differences were necessary to accommodate the difference in area of the two municipalities and because in Ponce many of the affected areas were contiguous over a larger area than those in Florida.

In Florida, the first reported case of dengue had a date of onset of symptoms on June 21, 1991 (week 1, fig. 6a). No additional cases were reported until 5

Table 1. Summary of number of cases reported to the CDC from the municipalities of Florida and Ponce, Puerto Rico

	Florida	Ponce
Period of georeferencing	January 1991 - December 1992	June 1994 - May 1995
No. cases reported to CDC	466	620
No. cases:		
georeferenced	377	495
negative for dengue	40	107
addresses outside area	37	4
no address	12	14
Percent of possible dengue cases georeferenced	97% (377 of 389)	97.3% (495 of 509)

Table 2. Summary of municipality and demographic characteristics of dengue cases georeferenced in Florida and Ponce, Puerto Rico

	Florida	Ponce
Area	26 km^2	300 km^2
1990 Census		
Population	8,689	187,749
Sex		
Males	4,243 (48.8%)	90,094 (48.0%)
Females	4,446 (51.2%)	97,655 (52.0%)
Age		
< 15 years	2,500 (28.8%)	54,503 (29%)
15-24 years	1,550 (17.8%)	33,269 (17.7%)
> 25	4,639 (53.4%)	99,977 (53.3%)
Dengue cases georeferenced		
Period of georeferencing	June 1991 - May 1992	June 1994 - May 1995
Total number of cases	294	495
Sex		
Males	136 (46.3%)	240 (51.5%)
Females	158 (53.7%)	255 (48.5%)
Age		
≤ 15 years	151 (51.4%)	203 (41.0%)
16-30 years	90 (30.6%)	150 (30.3%)
> 30 years	53 (18.0%)	142 (28.7%)
Laboratory results		
positive	139 (47.3%)	115 (23.3%)
indeterminate	155 (52.7%)	380 (76.8%)

weeks later when three new cases were observed in 2 of 18 areas (see week 6, fig. 6a). After one new case the following week, dengue incidence increased dramatically during week 8 (August 4-10, 1991) to 15 cases distributed among six neighborhoods over a wide geographic area (fig. 6b). During the following 7 weeks the rate of transmission remained high (11 to 18 cases per week; see fig. 6c for an example) with the disease spreading to an additional nine neighborhoods at a rate of zero to three new urbanizations per week (fig. 5). In the affected urbanizations, focal transmission continued. Incidence was highest between September 29 and October 5, 1991, when 39 cases were reported (fig. 6d). The rate of transmission decreased for the remainder of 1991, with only a few new cases. Of the 18 areas affected in Florida, eight had more than 10 reported dengue cases. These areas were the larger, most heavily populated neighborhoods. In most of these areas dengue cases were detected each week after the virus was introduced but in a few neighborhoods, 1 to 3 weeks passed between outbreaks of new cases.

In contrast, the progression of dengue through Ponce was characterized by the sporadic occurrence of cases until September 1994 when the incidence of dengue began to increase (fig. 5b). All cases reported during this period were separated by a minimum of 1 km (figs. 7a, 7b). During week 18 (September 25 - October 1, 1994) of the transmission season, dengue incidence began to increase and the new cases remained dispersed among many urbanizations (fig. 7b). By the end of October, however, new cases started to appear at sites in close proximity to recent or concurrent cases (week 23, fig. 7c). Overall, dengue activity in Ponce began and peaked about 8 to 10 weeks later in the transmission season than that observed in Florida. Peak dengue incidence occurred in Ponce between week 27 (November 27 - December 3, 1994) through week 29 (December 11-17, 1994), affecting approximately 94 urbanizations and rural areas (week 29, fig. 7d). Dengue incidence then decreased for the remainder of the transmission season. By the final week of the season approximately 113 urbanizations were affected and only seven had no reported cases (fig. 7e).

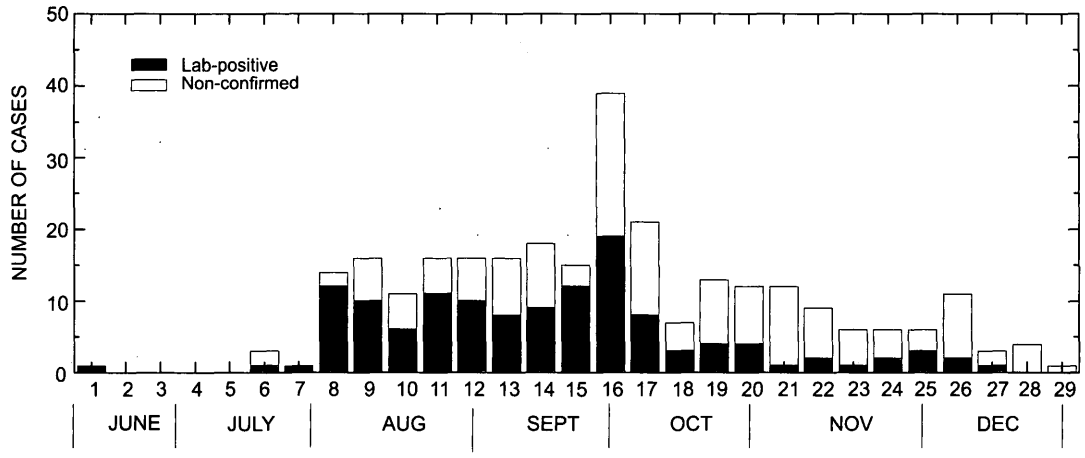

Figure 5a. Weekly reported dengue cases from Florida, Puerto Rico, between June 16, 1991, and January 4, 1992. Week numbers represent consecutive weeks starting with June 16-22, 1991 (week 1) through December 29, 1991 - January 4, 1992 (week 29).

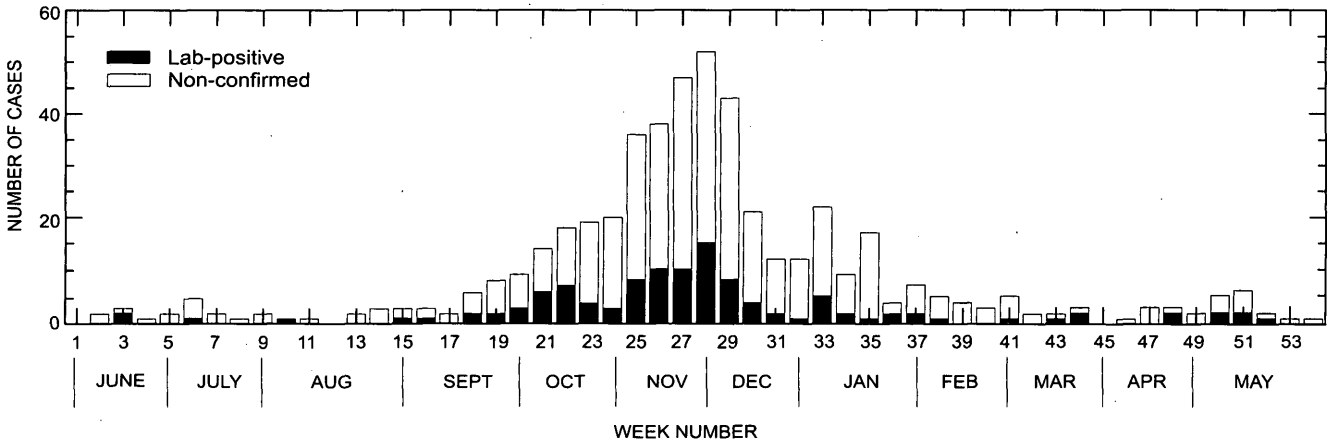

Figure 5b. Weekly reported dengue cases from Ponce, Puerto Rico, between May 29, 1994, and June 4, 1995. Week numbers represent consecutive weeks starting with May 29 - June 4, 1994 (week 1) through May 27 - June 2, 1995 (week 54).

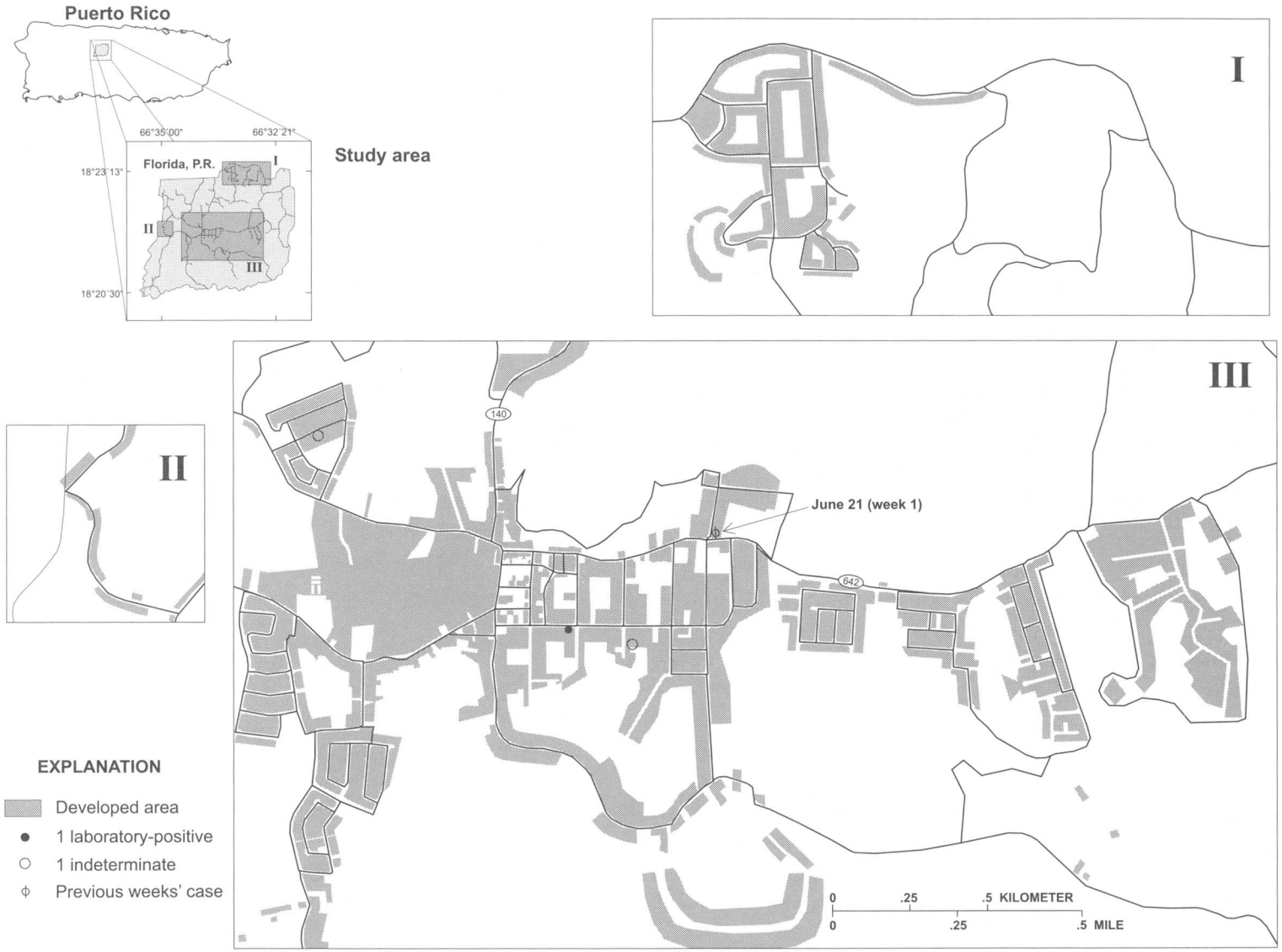

Figure 6a. Incidence of reported dengue cases by residential address locations in three regions of Florida, Puerto Rico, highlighting weeks illustrating the progress of the epidemic, week 1 (June 16-22, 1991) and week 6 (July 21-27, 1991).

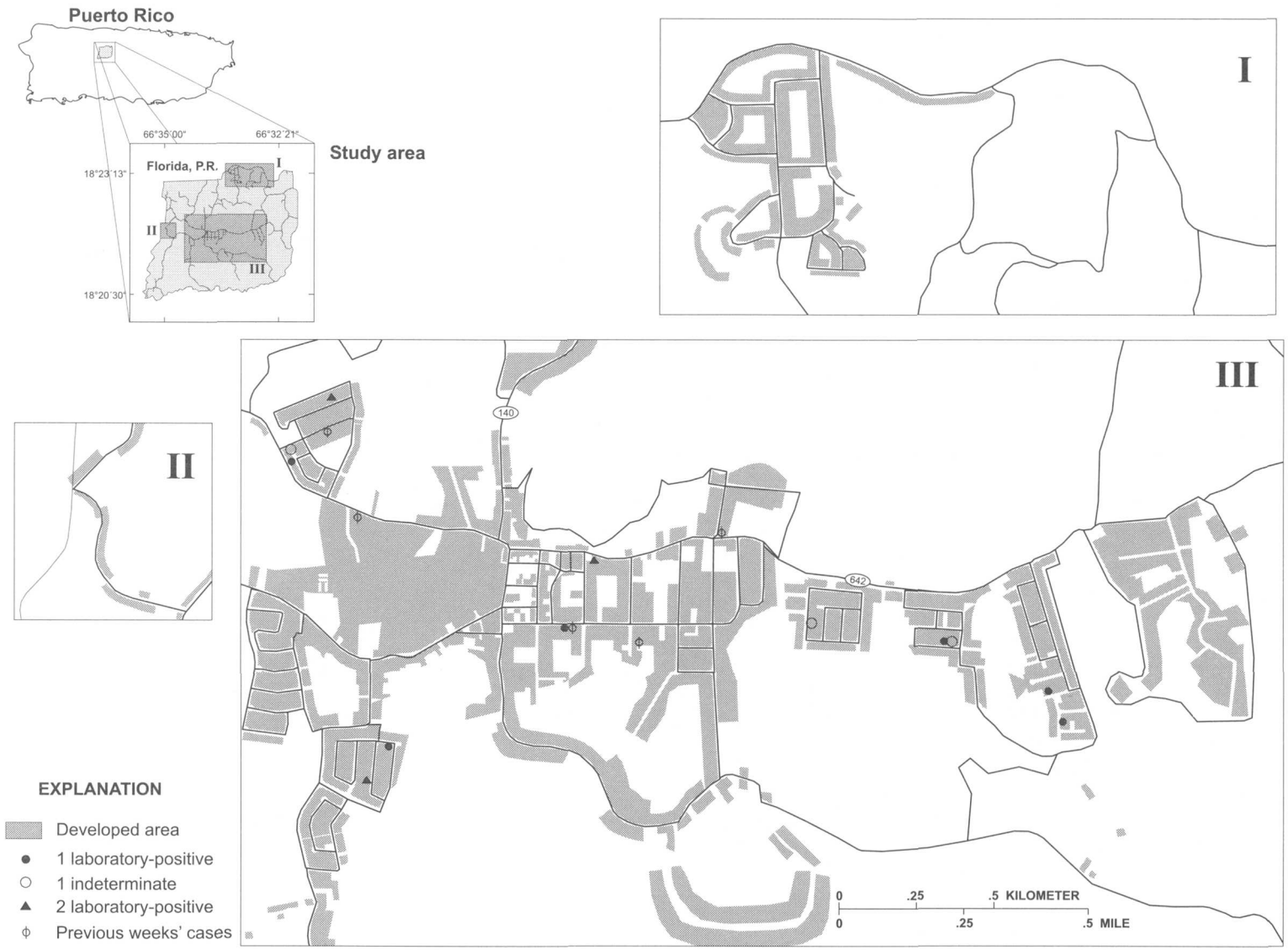

Figure 6b. Incidence of reported dengue cases by residential address locations in three regions of Florida, Puerto Rico, highlighting weeks illustrating the progress of the epidemic, week 8 (August 4-10, 1991).

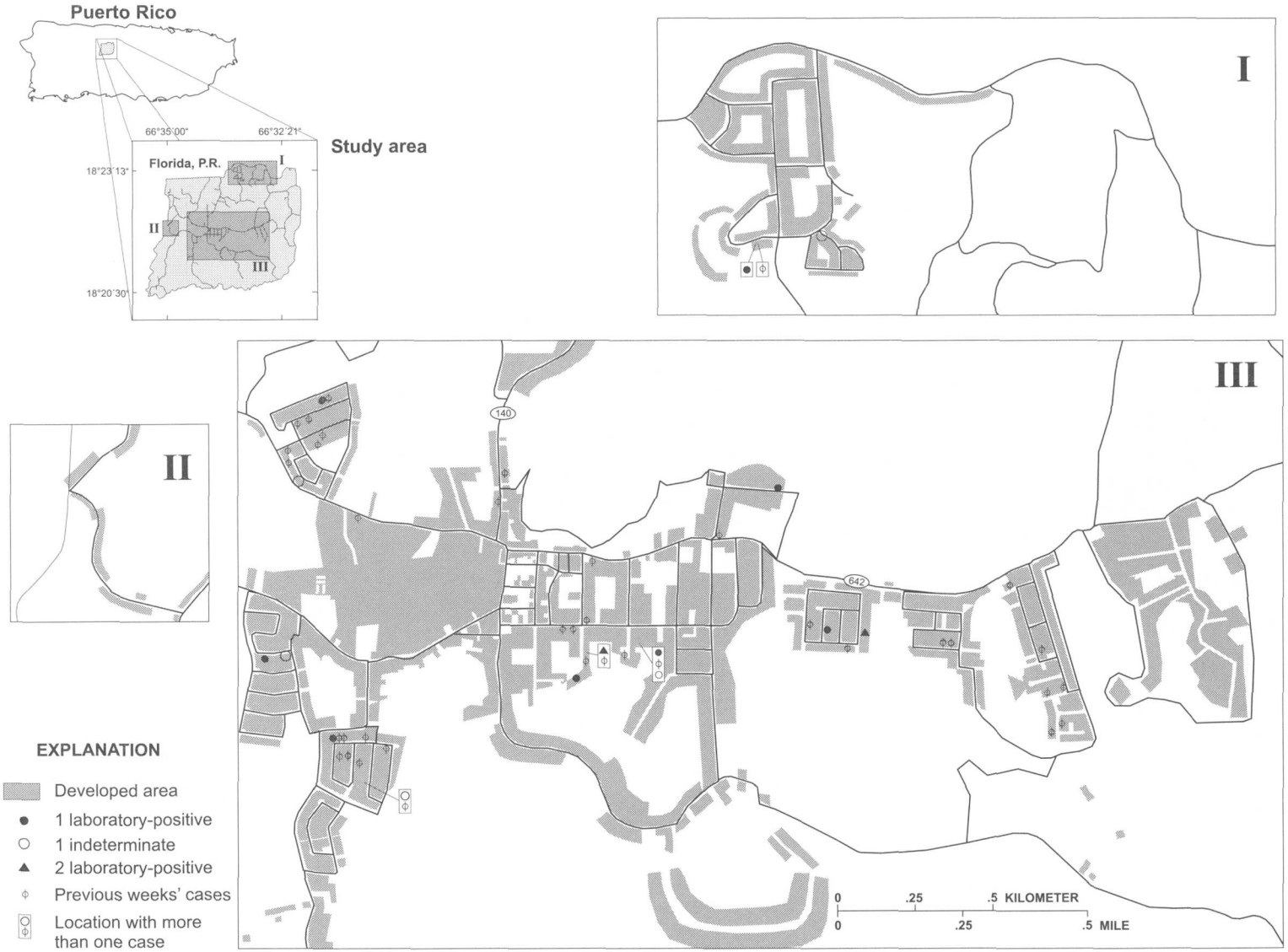

Figure 6c. Incidence of reported dengue cases by residential address locations in three regions of Florida, Puerto Rico, highlighting weeks illustrating the progress of the epidemic, week 11 (August 25-31, 1991).

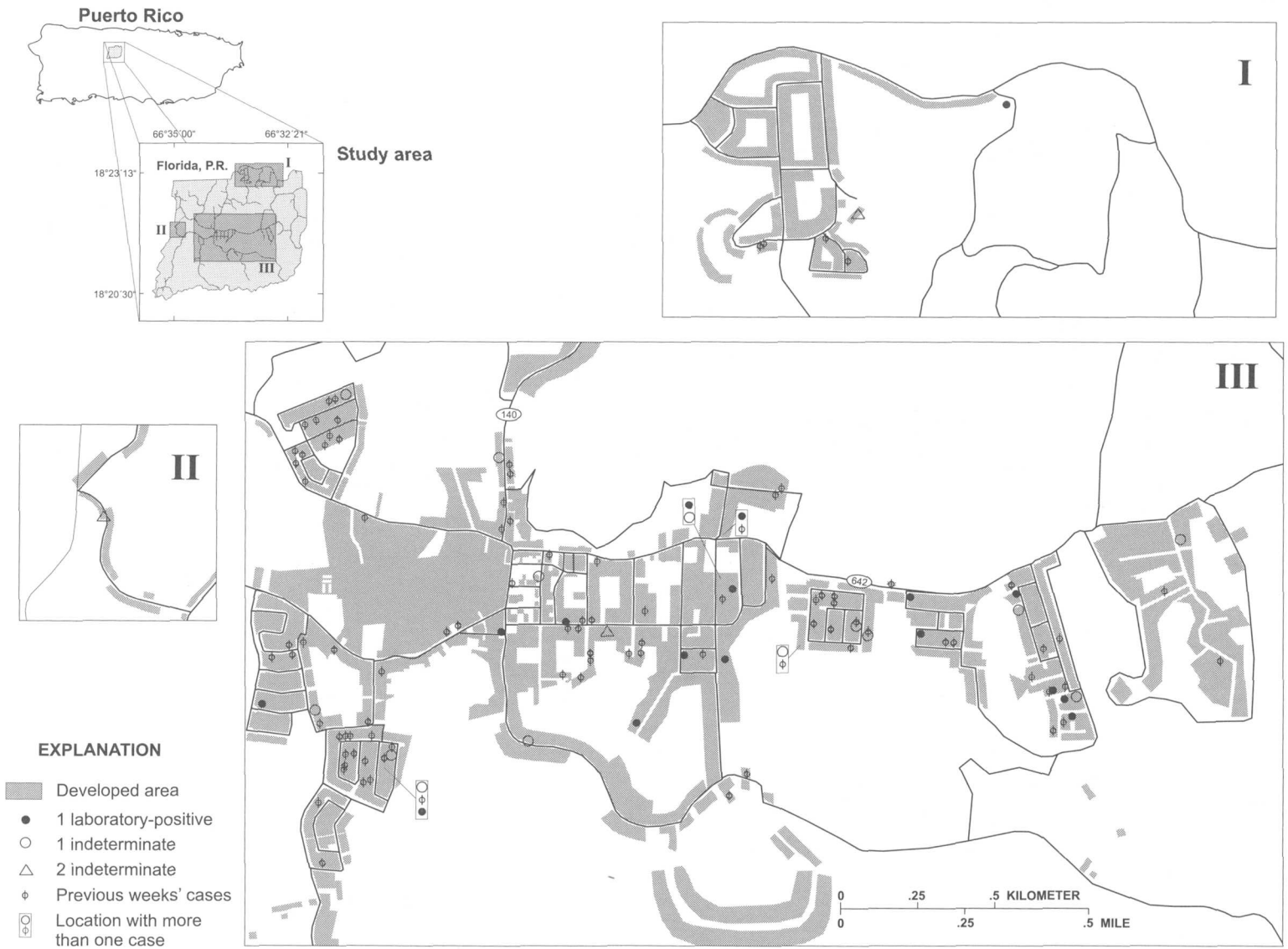

Figure 6d. Incidence of reported dengue cases of residential address locations in three regions of Florida, Puerto Rico, highlighting weeks illustrating the progress of the epidemic, week 16 (September 29 - October 5, 1991).

Figure 7a. Incidence of reported dengue cases by residential address locations in the urban area of Ponce, Puerto Rico, highlighting weeks illustrating the progress of dengue transmission, week 9 (July 24-30, 1994).

Figure 7b. Incidence of reported dengue cases by residential address locations in the urban area of Ponce, Puerto Rico, highlighting weeks illustrating the progress of dengue transmission, week 18 (September 25 - October 1, 1994).

Figure 7c. Incidence of reported dengue cases by residential address locations in the urban area of Ponce, Puerto Rico, highlighting weeks illustrating the progress of dengue transmission, week 23 (October 30 - November 5, 1994).

Figure 7d. Incidence of reported dengue cases by residential address locations in the urban area of Ponce, Puerto Rico, highlighting weeks illustrating the progress of dengue transmission, week 29 (December 11-17, 1994).

Figure 7e. Incidence of reported dengue cases by residential address locations in the urban area of Ponce, Puerto Rico, highlighting weeks illustrating the progress of dengue transmission, week 53 (May 28 - June 3, 1995).

When the seasonal increase in dengue incidence occurred in Ponce, the observed pattern of dengue cases was similar to that of Florida, only on a larger scale. This is illustrated by comparisons made with nearest case pair analysis. Frequency distributions of the time and distance intervals separating nearest case pairs for both Florida and Ponce are shown in figures 8 and 9, respectively. These results indicate that there is slightly more clustering of cases in time in Florida than in Ponce, 54 percent compared with 41 percent of nearest case pairs within 10 days of each other, respectively. The lower percentage of dengue case pairs separated by less than 10 days in Ponce reflects the sporadic transmission seen early in the season. Spatially, most of the nearest case pairs (about 90 percent) occurred within 500 m of each other in Florida compared with almost 2.4 km for the same percentage in Ponce. Once the dengue season in Ponce was well underway, areas of focal transmission were also observed.

The municipalities of Florida and Ponce represent communities where dengue transmission was epidemic and endemic, respectively. During the 1991 dengue outbreak in Florida, dengue incidence increased to levels 30 times higher than the average incidence recorded in previous years. In contrast, dengue incidence in Ponce remained about the same as the average for the two previous seasons (1.4 cases per 1,000 people). These differences in transmission dynamics may explain, in part, the differences in dengue case pattern observed, but they also bring into question the interpretation of indeterminate laboratory results in the two municipalities. The previously low levels of dengue in Florida indicate that the population was highly susceptible, and the ensuing levels of transmission in the community suggest that a high percentage of the cases with indeterminate laboratory results were indeed dengue. Conversely, in an area like Ponce where there were higher levels of endemic transmission, indeterminate results were more difficult to interpret.

Overall, the most striking characteristic of the Florida and Ponce transmission seasons were the rapid spread of the disease throughout the entire municipality. No directional movement of dengue virus could be detected during either transmission season. For the 1991-92 transmission season in Florida, the Barton and David test identified 23 temporal clusters of dengue cases. No difference between the geographic centroids of these clusters was detected ($Z = 0.344$, $P > 0.05$), indicating that the spatial patterns for the temporal clusters were not significantly different. This indicates that there was no change in the overall pattern of cases through the course of the epidemic, providing some statistical support for the visual observations. Another important observation about the Florida outbreak is that, with very few exceptions, once a case of dengue had been reported in a particular neighborhood, transmission in that area would continue for an extended period of time (more than 6 weeks).

There are several plausible explanations for the wide geographic distribution (in both municipalities) of dengue cases early in the transmission season. The pattern may be a result of the insensitivity of the dengue surveillance system, especially during the early stages of an epidemic. Previous estimates of asymptomatic disease ranging from 43 to 53 percent are based on serosurveys carried out during dengue outbreaks (Likosky and others, 1973; Waterman and others, 1985; Rodríguez-Figueroa, 1995). Information about these incidence rates in areas with endemic transmission are not available. When a virus is introduced into a community (such as in Florida) or exists at very low levels (the off-season in Ponce) an accurate picture of disease progression during the early part of the transmission season is likely to be obscured. The dengue surveillance literature commonly describe a characteristic "lag phase," ranging from a few weeks to several months, when few cases are reported due to low levels of suspicion by physicians (Gubler, 1989a; Gubler and Casta-Vélez, 1991). Because of the high mobility of the Puerto Rican population, working and traveling outside their municipality of residence, dengue virus can be introduced into a municipality many times over a wide geographic area during a transmission season. Mosquito dispersal could also contribute to the movement of virus within the community, since *Ae. aegypti* may be capable of movement over distances of several kilometers (Reiter and others, 1995). However, the natural barriers between these urbanizations, and the fact that only a small percentage of mosquitoes survive the extrinsic incubation period, preclude the simultaneous appearance of cases in geographically separated urbanizations. The role of *Ae. aegypti* in the dispersal of virus within urbanizations, however, is likely to be significant.

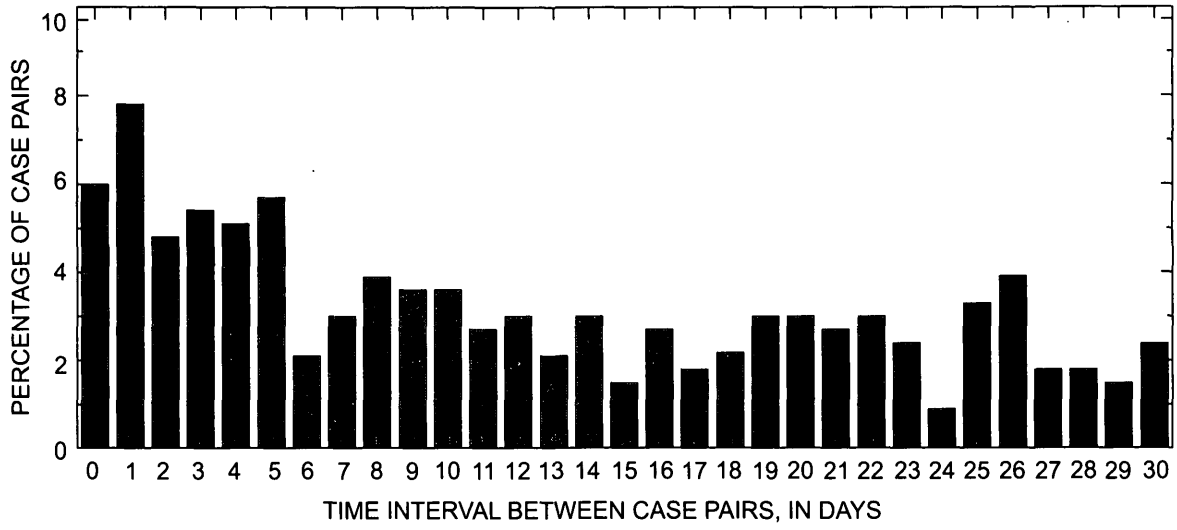

Figure 8a. Frequency distribution of nearest dengue case pairs in time (Florida, Puerto Rico).

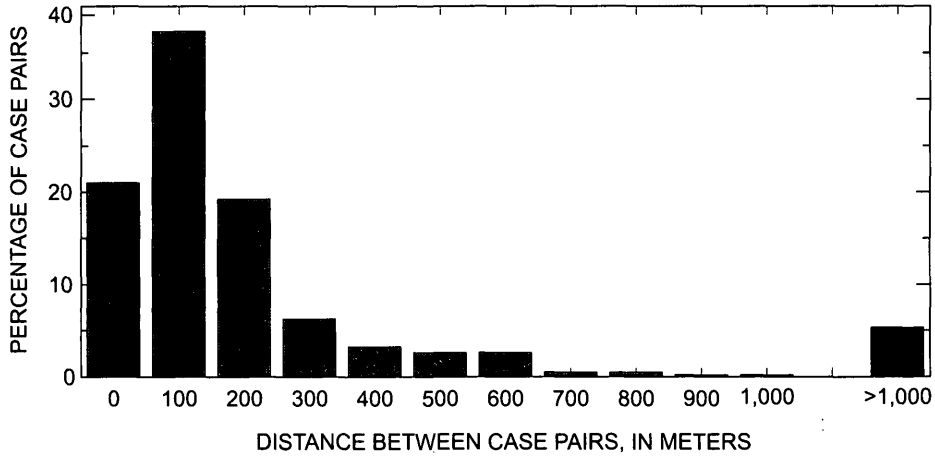

Figure 8b. Frequency distribution of nearest dengue case pairs in space (Florida, Puerto Rico).

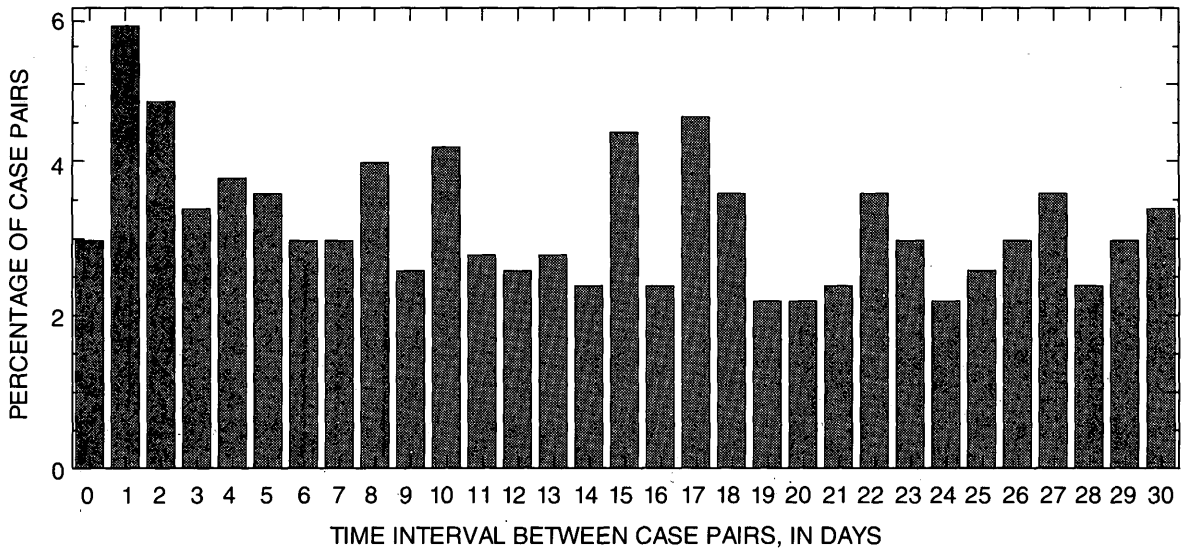

Figure 9a. Frequency distribution of nearest dengue case pairs in time (Ponce, Puerto Rico).

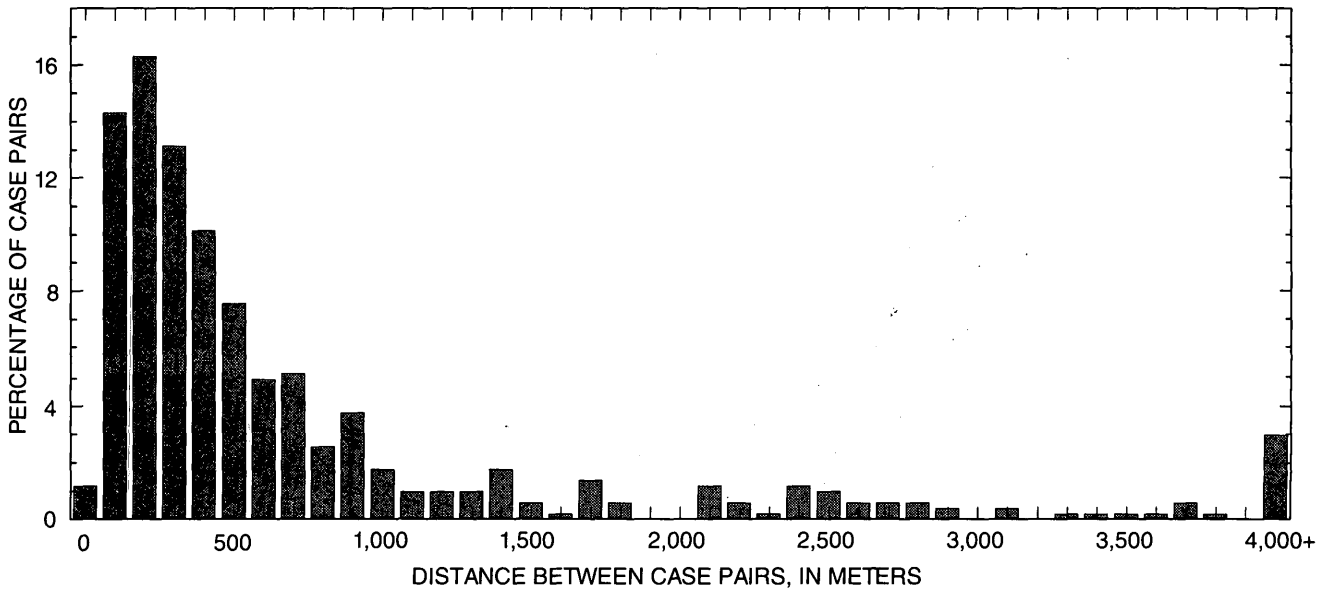

Figure 9b. Frequency distribution of nearest dengue case pairs in space (Ponce, Puerto Rico).

CHARACTERIZATION OF DENGUE CASE CLUSTERING PATTERNS AND FLORIDA SPATIAL ANALYSIS

In Florida, dengue cases clustered inside individual houses (fig. 10). For the June 1991 - May 1992 transmission season, dengue cases were reported in 217 houses. Of these, 56 houses (25.8 percent) had between 2 and 6 reported cases, with 45.2 percent, 8.4 percent, and 20.2 percent of the cases occurring within 5 days, 6 to 10 days, and 10 to 30 days, respectively, of the subsequent cases within the same house. The remaining 26.2 percent of the cases occurred more than 30 days apart. In many instances, a single **index case** could not be identified within the house (fig. 11a).

In contrast, only 7.3 percent of the houses (33 of 453) in Ponce had more than one reported case. There was some temporal clustering of cases; 33.3 and 10.3 percent of the subsequent cases occurred within 5 days and 6 to 10 days, respectively (fig. 11b). More significant, however, was the observation that 35.9 percent of the cases occurred more than 30 days apart (32-354 days), indicating that fewer household cases were related (that is, a case arising from a mosquito that was infected by another household member) to each other than observed in Florida.

To better describe the spatial clustering patterns of dengue cases, K-functions were calculated for the Florida case data. The K-functions for the Florida cases (all cases and laboratory-positive cases only) and lot locations weighted by the persons per household are displayed in figure 12. As distance increased from each of the 2,989 lots contained within Florida, the population increased rapidly over short distances (to 100 m) (fig. 4). If the human population was randomly distributed in Florida, the K-function would be a straight line well below the actual curve representing the population distribution (fig. 12). The slope of the K-function for all cases of dengue is similar to that of the population beyond 10 m, but at less than 10 m, the height of the case curve is much greater than the population curve. Since the minimum distance separating adjacent lots in Florida is 7 m, the higher K(d) values observed at 5 and 10 m implies significant clustering of dengue cases within households. Beyond the household, however, the distribution of cases was similar to the distribution of the population. For the laboratory-positive cases, clustering extended up to 15 m compared to 10 m in the combined group (fig. 12).

The K-function was used to compare sex, age, and reporting-status of the patient to identify patterns. When K-functions were stratified by sex, the observed clustering patterns were contradictory. For all cases, females clustered more than males (fig. 13a). In contrast, for laboratory-positive cases, more dengue in males was observed within 15 m of each other than for females. Beyond 15 m the patterns of no clustering are nearly identical (fig. 13b). Although the reasons for these differences are speculatives, further investigations could confirm or negate the following hypotheses: females are more likely to be infected at home than males or females are more likely than males to move between nearby households.

No distinctive clustering was observed by age-group for all cases (fig. 14a). For laboratory-positive cases, dengue in children less than 15 years old appeared to be more clustered over longer distances than for all of the cases (fig. 14b) to a distance of approximately 60 m.

A major concern about the inconsistency between the spatial analyses carried out for all cases and laboratory-positive cases alone is the possibility that the spatial distribution for indeterminate cases is different from that of laboratory-positive cases. These cases were included in the analysis because of the greater than 80 percent positivity rate observed during this dengue outbreak, indicating that at least 80 percent of the indeterminate cases were true dengue. There was a large temporal difference in the confirmation rate towards the end of the outbreak. Over-reporting often occurs later in the course of an epidemic (Klaucke, 1994) because of increased awareness of the disease. At the same time, confirmation rates may decrease because of increased confidence in clinical diagnoses by physicians who do not request additional tests, such as a paired serum sample to confirm the diagnosis.

Figure 10. Residential address locations where dengue cases were reported over the June 1991 - May 1992 transmission season in Florida, Puerto Rico.

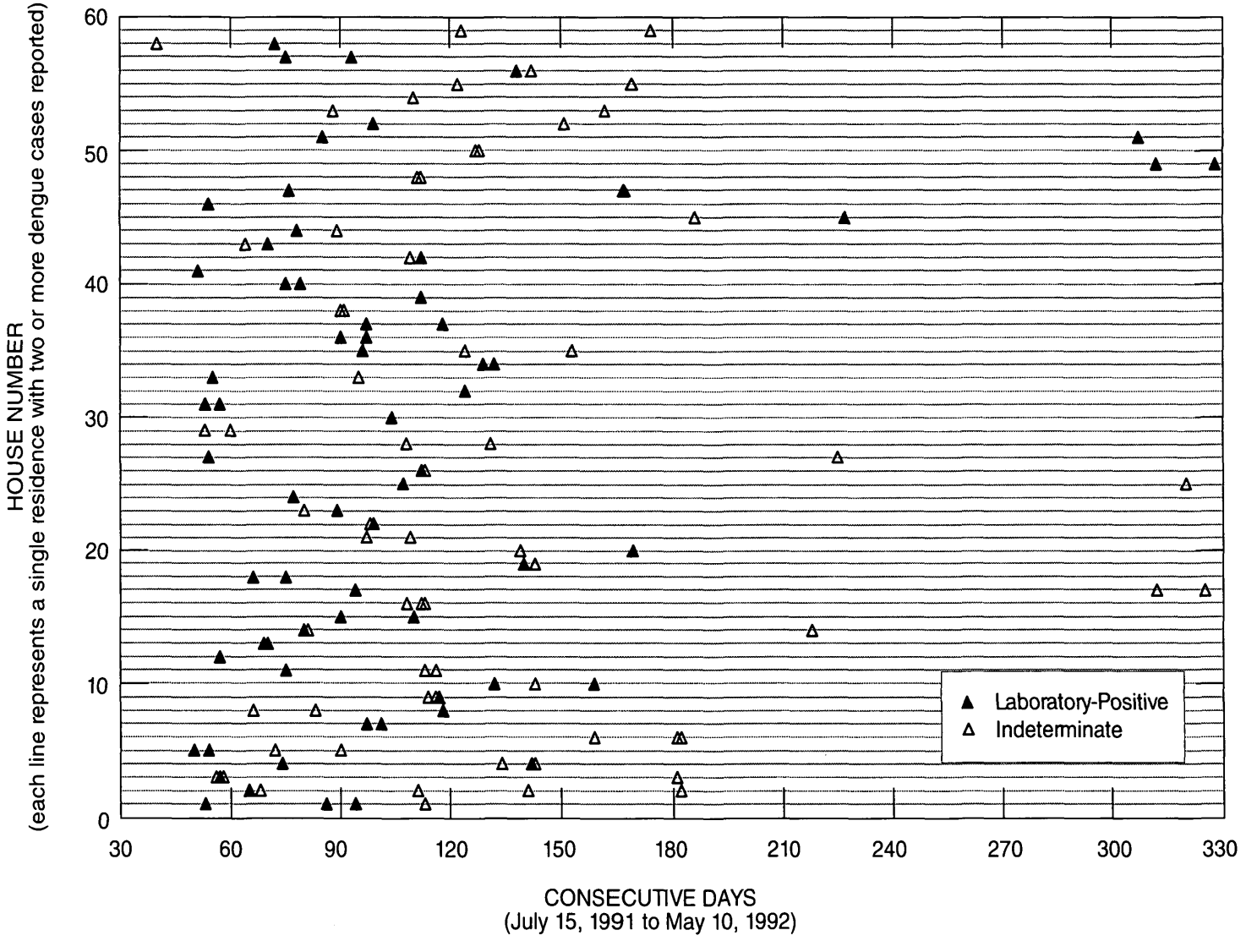

Figure 11a. Temporal distribution of reported dengue cases occurring in the same households in Florida, Puerto Rico, between July 15, 1991 to May 10, 1992.

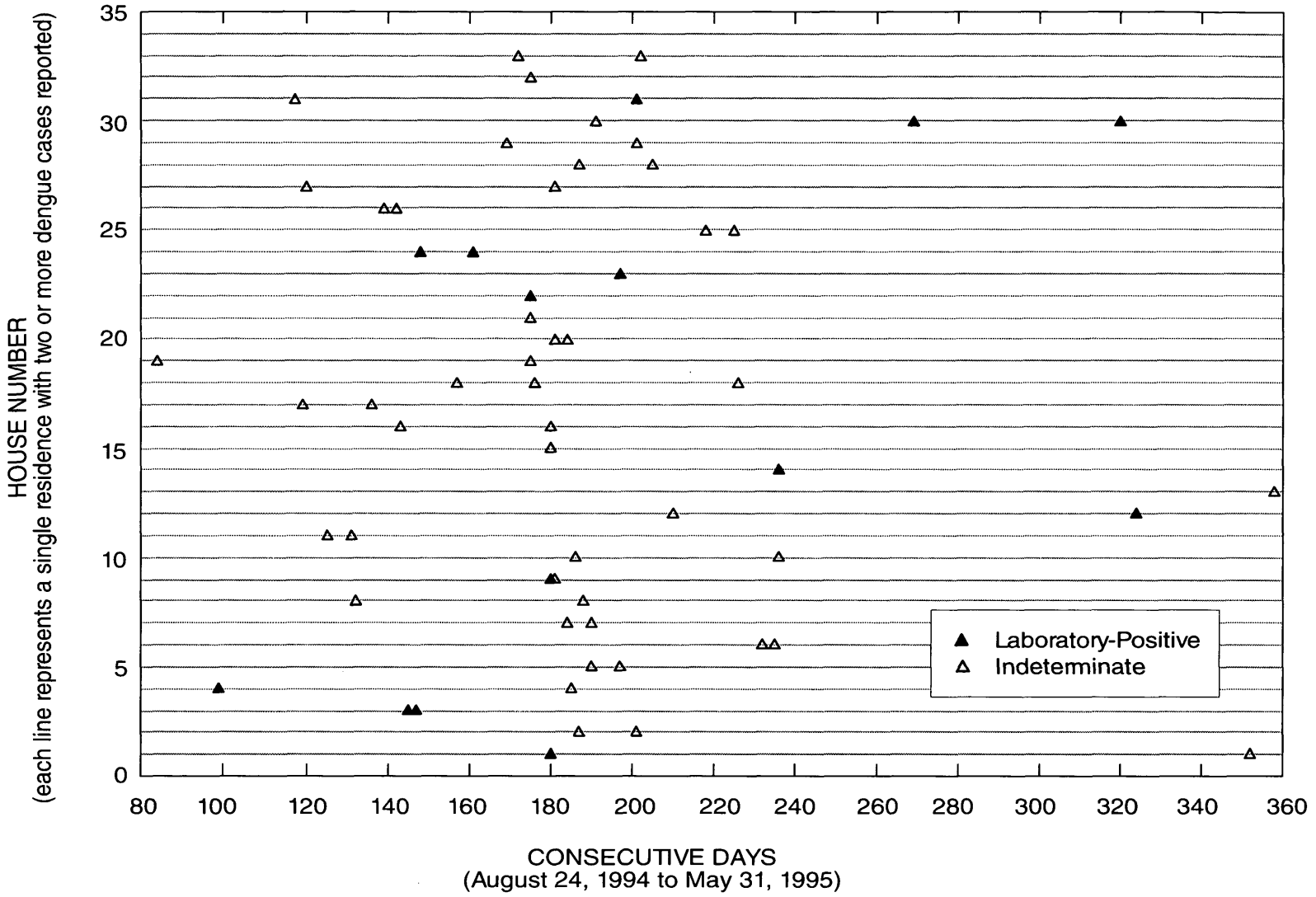

Figure 11b. Temporal distribution of reported dengue cases occurring in the same households in Ponce, Puerto Rico, between August 24, 1994 to May 31, 1995.

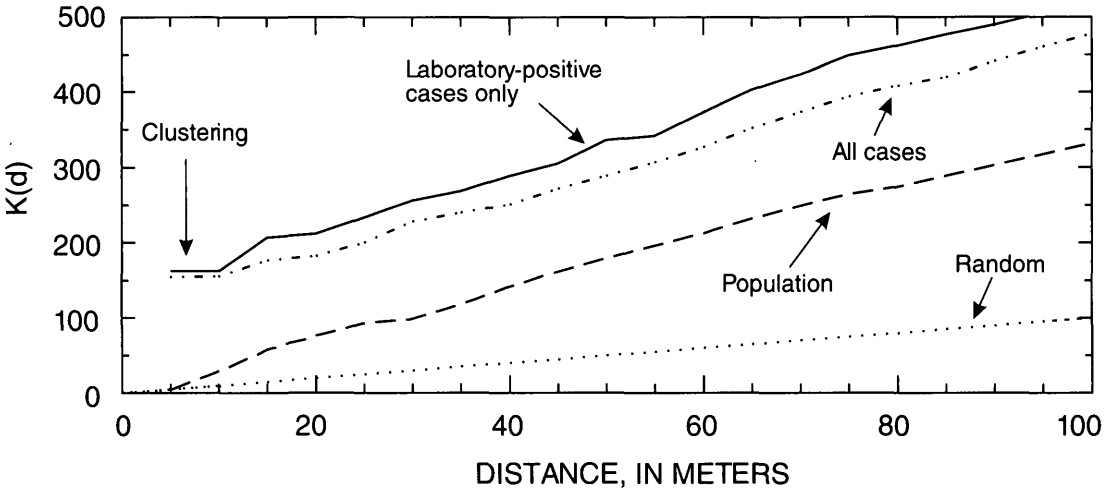

Figure 12. K-function for the location of laboratory-positive and reported (laboratory-positive and indeterminate) dengue cases and weighted (persons per household) lots for the June 1991 - May 1992 transmission season in Florida, Puerto Rico. K(d) was calculated for 5-m intervals.

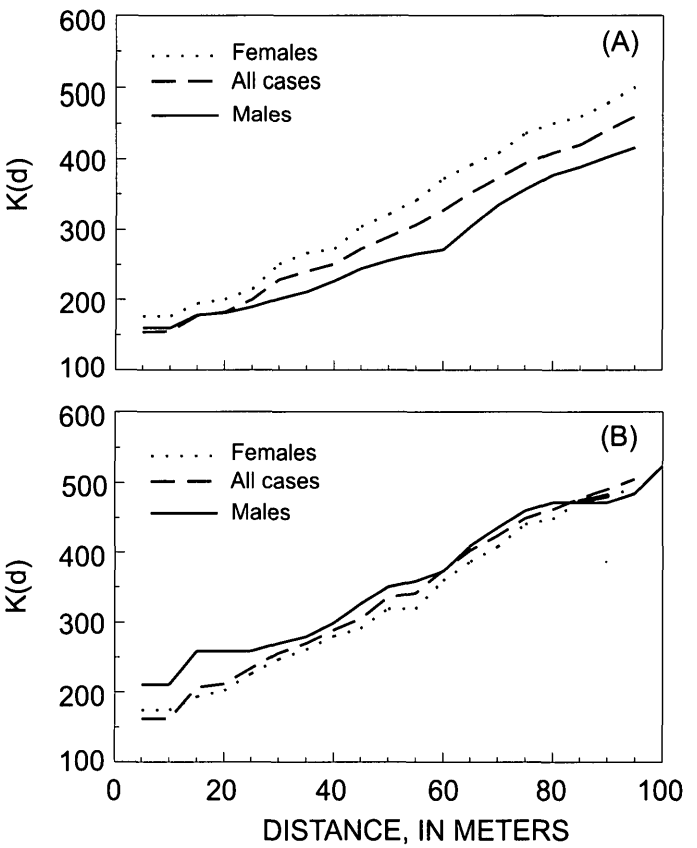

Figure 13. K-functions for male and female dengue cases reported from Florida, Puerto Rico, between June 1991 and May 1992. (A) All reported dengue cases (laboratory-positive and indeterminate), (B) Laboratory-positive cases only.

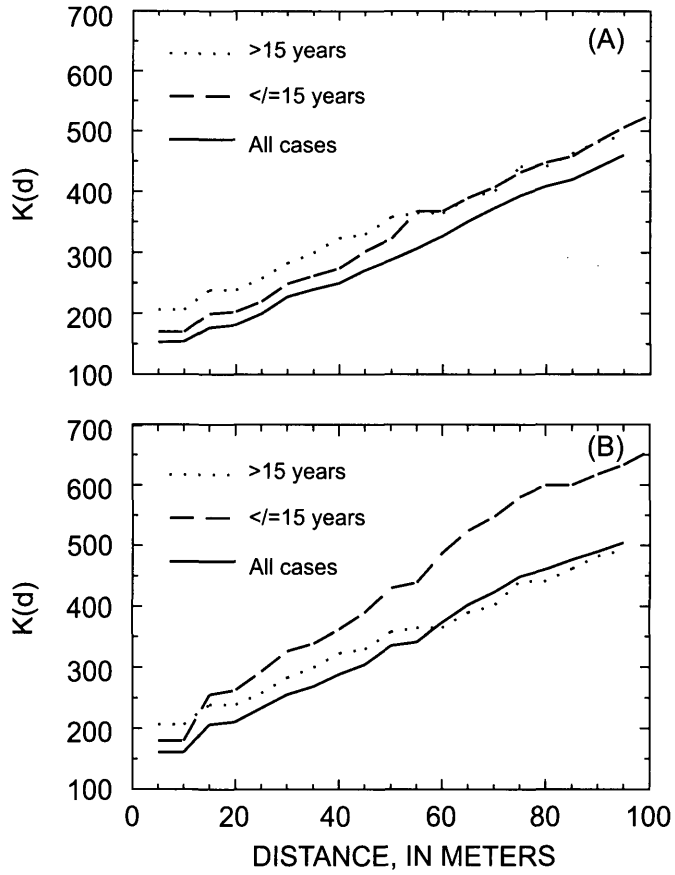

Figure 14. K-functions for the patterns of dengue cases less than or equal to 15 and greater than 15 years of age in Florida, Puerto Rico (June 1991 - May 1992). (A) All reported dengue cases (laboratory-positive and indeterminate), (B) Laboratory-positive cases only.

Based on the results of the K-function analysis, the Knox test was used to test the following hypothesis: that the spatial distance between cases is no greater than 5 m. Since a female *Ae. aegypti* mosquito begins oviposition approximately 3 days after taking her first blood meal, this number was used to define the time-space window tested. For 1991-92 transmission season, 29 case pairs occurred within 5 m and 3 days of each other, compared to an expectation of 5.43 case pairs, given the 40,470 pairs of possible interactions. Thus significantly more cases occurred within the 5-m, 3-day time-space window than would be expected by chance ($P < 0.0001$). These results were similar for laboratory-positive cases. Since dispersal of *Ae. aegypti* may be driven by oviposition as suggested by Reiter and others (1995), a 1- to 2-day blood-feeding period, possibly in the same house, followed by oviposition extending over several days would be expected. Thus, the hypothesis that clustering would extend to nearby houses after a period consistent with the gonotrophic cycle of the mosquito was tested. Significant clustering was observed at 25 m and 4 days and also at 35 m and 5 days. Using this same approach, significant clustering inside houses up to 10 days was observed. This illustrates the need to use only pre-planned comparisons with this technique.

The local K-functions for three representative case locations and a special dengue case site are shown in figure 15. A commonly observed pattern for a case occurring in a house with multiple cases, shows a distinct cluster up to 5 m and that the next closest case was between 25 to 30 m away (fig. 15a). Overall, the case curve beyond 5 m exhibits a slope similar to the population distribution surrounding the given household and is similar to the general Florida pattern

(fig. 12). In contrast, for another case from a household having no further cases, the nearest case during the epidemic was 35 m away (fig. 15b). Only one of 294 cases (fig. 15c) that had a K-function value greater than 2 standard deviations from the mean at 100 m could be identified. This single location showed clustering over the population between 65 to 100 m, but would not be considered a "hot spot" simply because in any normal distribution a few reasonable outliers would be expected. Again, this implies that the only "hot spots" were those households where multiple cases were reported.

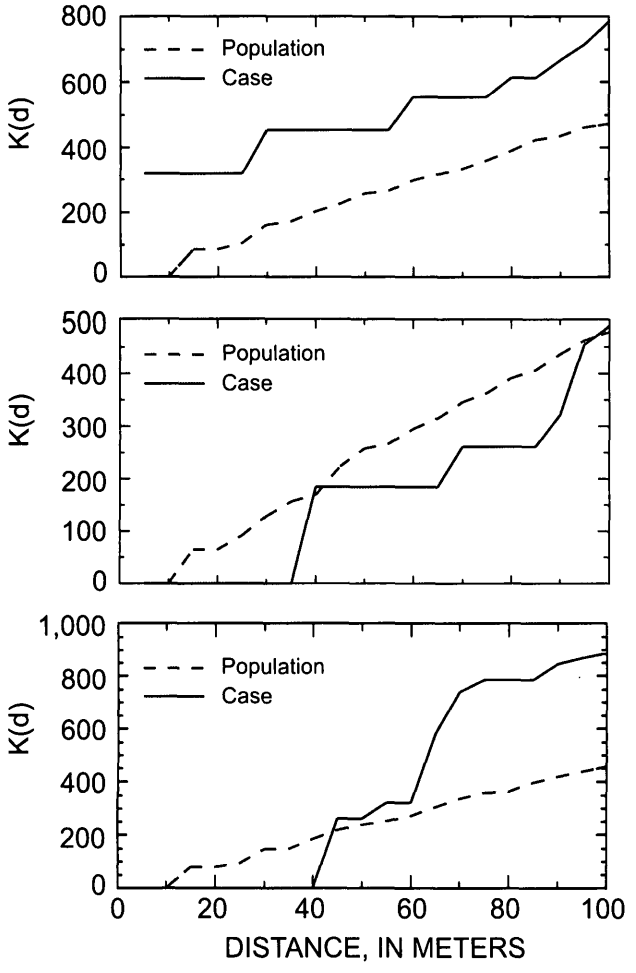

Figure 15. Local K-functions of three representative case locations in Florida, Puerto Rico.

Clustering of dengue cases inside houses has been described previously (Halstead and others, 1969; Likosky and others, 1973; Rodríguez-Figueroa, 1995). Halstead and others (1969) noted that the onset dates of family members hospitalized with DHF were often separated by only a few days. In Florida, the authors were able to demonstrate clustering of dengue cases inside houses and at short time intervals (3 days). Histologic studies (Scott and others, 1993a, b) and field observations (MacDonald, 1956; Gould and others, 1970) have demonstrated that *Ae. aegypti* commonly blood-feeds multiple times during a single gonotrophic cycle. Epidemiologically, this behavior implies that clusters of dengue patients in the same household with a similar date of onset of illness (Waterman and others, 1985; Gubler, 1992) had occurred in Florida, accompanied by the rapid and often explosive spread of dengue (Gubler, 1992). Two additional factors could contribute, in part, to the high degree of household clustering. If *Ae. aegypti* abundance was high, time-space clusters may also be a result of individuals infected by different mosquitoes in the same house. Entomological studies conducted in Florida during August 1991, indicated that the average number of *Ae. aegypti* per person was 2.3 (Centers for Disease Control and Prevention, unpublished data) but that the risk of dengue infection rose with increasing mosquito density (Rodríguez-Figueroa, 1995). The hypothesis that case clusters result from infection by different mosquitoes is extremely sensitive to the daily survival of *Ae. aegypti*, estimated between 66 and 88 percent (Sheppard and others, 1969), and assumes that those mosquitoes would remain in the same house for 2 to 3 gonotrophic cycles, since an infected mosquito needs to survive a minimum of 8 days to transmit dengue virus. For this reason, multiple feeding behavior remains the simplest explanation for household clustering of dengue cases.

Serial plots of dengue cases revealed temporal clusters, but spatial clusters beyond the household were not in evidence (figs. 5, 6). The K-function analysis revealed a spatial pattern of cases that mirrored the pattern of the population distribution beyond about 10 m or the individual house. In addition, no "hot spots" were identified outside household clustering.

This pattern of household clustering suggests two important possibilities: wider level clustering (for example, block) was not detected due to the insensitivity of the surveillance data or because virus dispersal mechanisms, whether by mosquito, human, or both, are highly efficient. As mentioned previously, it is probable that less than half of the dengue infections in Florida are detected by the surveillance system (Rodríguez-Figueroa and others, 1995).

Analysis at the TIGER census block level had a resolution of 50 m and yielded the results summarized in figure 16. The straight line represents a spatial pattern indicating that the population of Florida is distributed at random. Note that at this scale the pattern of cases and the actual distribution of the population follow the same trend. The K-function for cases is higher than that of the population indicating slightly more clustering of cases than the population, especially at distances up to 650 m and then from 800 to 1,000 m. No considerable clustering or extraordinary variations are evident. For males and females in the population (not shown), the pattern was nearly identical to the population pattern. Persons per household for the 83 TIGER census blocks analyzed does appear to cluster to about 400 m. Accordingly, the blocks, which on average are about 200 m from each other, tend to group spatially by the population density of households. That is, blocks having high population density, such as those in the center of the region, are within a relatively short distance of other blocks with high population densities.

Although georeferencing of dengue cases might be more practical at the census block level, analysis at this level was probably not appropriate because patterns at a smaller geographic scale were obscure.

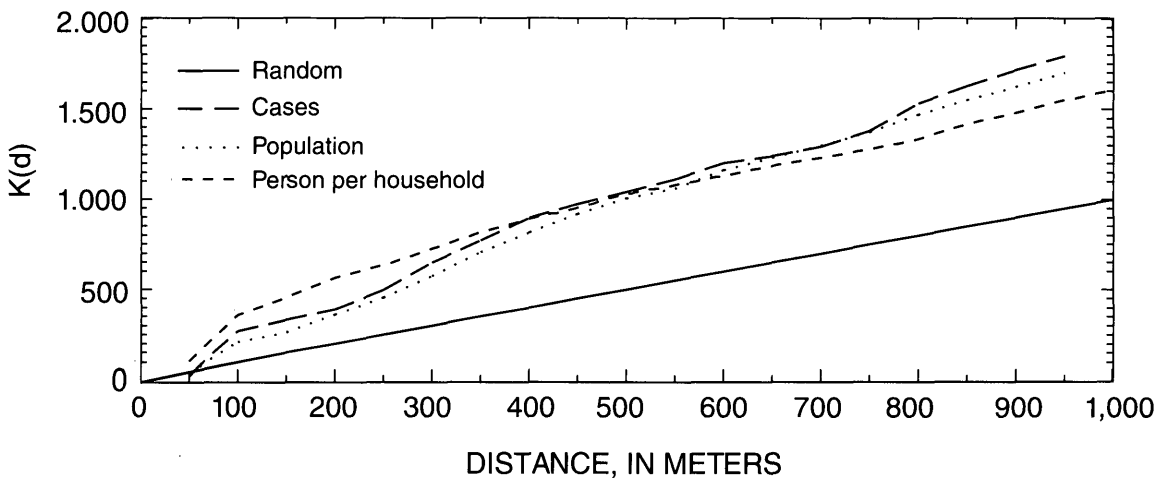

Figure 16. K-function for dengue incidence, population size and density by census blocks in Florida, Puerto Rico (June 1991 - May 1992).

MOVEMENT OF DENGUE SEROTYPES THROUGHOUT PUERTO RICO

During the period between 1988 and 1994, three dengue serotypes were circulating on the island of Puerto Rico. Monthly maps were generated for each dengue transmission season during the study period to observe the movement of virus throughout the island (appendixes A to F). The relative abundance of each serotype varied with transmission season. For example, in the first half of the 1988-89 dengue transmission season, dengue-4 was the most prevalent serotype. In early 1989, dengue-2 became more prevalent, slowly replacing dengue-4 during the 1989-90 transmission season. During the 1990-91 dengue transmission season, dengue-2 was the most commonly isolated serotype. The 1991-92 season was characterized by the disappearance of dengue-4, the peak of dengue-2 and the clear re-emergence of dengue-1. By 1993, dengue-1 had become the predominant dengue serotype. Although, all three serotypes were persistent in Puerto Rico, one generally appeared to predominate during any one season. This pattern was consistent with what one might expect in the case of intense transmission and a reduction of the pool of susceptible humans. The observed dengue serotype movements indicated that the greater San Juan Metropolitan area may be the site of endemic transmission or virus serotype maintenance. Alternatively the close proximity of the CDC San Juan Laboratories to the Metropolitan area hospitals may increase the possibility of virus isolation in that area. Isolates were consistently made from the area year round and cases then spread to other parts of Puerto Rico. The high mobility of the Puerto Rican population, may support this type of pattern.

GIS AND DENGUE SURVEILLANCE

One of the more practical objectives of this project was to evaluate the potential for incorporating GIS into the current dengue surveillance program. Currently, the only geographic information available for all dengue cases reported to CDC in Puerto Rico is the municipality where the person reporting the case lives. This is clearly an inappropriate scale for studying environmental (for example, weather) and biological (for example, *Ae. aegypti* flight and feeding behavior) factors. Although, the display capabilities of a GIS made visualization of serotype movement possible, these maps can be generated in other software packages without GIS capabilities such as Epi-Info (a word-processing, database, and statistics program for epidemiology produced by CDC) (Centers for Disease Control, 1993). The additional expense and expertise necessary for GIS software would be justified only by its spatial analysis capabilities. The climate analysis, however, illustrated that for dengue, case data must be georeferenced at a finer scale than the municipality to carry out meaningful spatial analysis.

Results of this study demonstrated the utility of a GIS if dengue case data were georeferenced to the level of a household. Adding a geographic component to the current system would be very useful for identifying "hot spots" dengue transmission. If exploratory spatial analysis were conducted at regular intervals, small areas could be studied retrospectively so that detailed questioning on the movement patterns of the affected individuals could be conducted. Also, once an outbreak is identified, additional entomological and serological data could be collected and used to compare the clustering and dispersal patterns of *Ae. aegypti* to those of dengue cases.

At present, there are a number of significant obstacles to incorporating a GIS into the current dengue surveillance program in Puerto Rico. These obstacles include the collection of accurate georeferenced address information and a lack of availability of 1:2,000 scale maps for all of Puerto Rico. The resources necessary to georeference the cases for Florida and Ponce were extensive (approximately 3.4 person-hours per georeferenced case). This time could be reduced if an address georeferencing system consisting of an address database containing the geographic coordinates for each residence/address on Puerto Rico could be developed. Currently, the only source of base maps at a 1:2,000 scale for Puerto Rico is the Puerto Rico Planning Board. However, these maps would require considerable revision and updating to be useful in a GIS dengue surveillance system database. TIGER line files available from the U.S. Census Bureau are inadequate because they are developed from USGS maps at a 1:20,000 scale that do not include local streets. An alternative, would be the use of a

differential GPS to obtain the geographic coordinates for each case. Furthermore, accurate and up-to-date base maps would still need to be developed to determine the location of the population in these areas.

Even if an address georeferencing system were available for Puerto Rico, the input of address data would need to be in a standardized and accurate form. At present, addresses are not included in the computerized CDC surveillance database. If a GIS were incorporated into the CDC surveillance system, the address variable would need to be included and more emphasis given to its collection. As a research tool, however, GIS has many potential applications for exploratory spatial analysis.

SUMMARY AND CONCLUSIONS

The spread of the dengue virus during the 1991-92 transmission season in Florida, P.R., (epidemic) and the spread during the 1994-95 dengue transmission season in Ponce (endemic) were similar in that the virus advanced rapidly, and cases appeared to be widely distributed throughout each municipality during the early part of the transmission season. This may be attributed to a widespread movement of the virus within the community by humans, mosquitoes, or both.

The rapid geographic spread of dengue was most evident at the level of individual houses. Significant dengue case clustering was identified at very short distances (most likely within households) over short time periods (3 days or less). This was probably a result of multiple feeding behavior on the part of the *Ae. aegypti* mosquito. However, beyond the level of the individual house, in general, the cases have spatial pattern characteristics that resemble the population pattern as a whole. The absence of wider level clustering is also indicative of widespread movement of the virus.

The results indicate that focal spraying and other area-limited responses against houses where cases are reported to control the spread of dengue is unlikely to be effective because of the rapid temporal and spatial expansion of the disease. Instead, at the first sign of dengue activity, municipality-wide measures need to be implemented. Although a dengue surveillance database managed within a GIS would add many useful elements to the surveillance system, it will not be practical until a reliable address georeferencing system is available for Puerto Rico.

At the macrogeographic level, three dengue serotypes were circulating in Puerto Rico between June 1988 and May 1994. However, the relative abundance of each serotype varied with transmission season. Dengue-4, the predominate serotype during the 1988-89 dengue transmission season, declined in subsequent years and was replaced by the dengue-2 serotype by 1991. During the 1991-92 season, dengue-4 occurrence became less prevalent and dengue-1 reemerged as the predominate serotype by 1993. Finally, variation of dengue transmission characteristics observed at the microgeographic level emphasizes the need to conduct additional dengue studies at this spatial scale that monitor dengue infections rather than reported cases and compare the clustering patterns of cases with that of its vector, *Ae. aegytpi*.

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GLOSSARY

The epidemiologic terms defined below are shown in bold in the text the first time they are used.

confounding: A situation in which the effects of two processes are not separated. The distortion of the apparent effect of an exposure on risk brought about by the association with other factors that can influence the outcome.

epidemic: The occurrence in a community or region of cases of an illness with a frequency clearly in excess of normal expectancy.

endemic: The constant presence of a disease or infectious agent within a given geographic area; it may also refer to the usual prevalence of a given disease within such an area.

extrinsic incubation period (EIP): The period between entry of the infectious agent into the vector and the time at which the vector becomes infective.

herd immunity: The immunity of a group or community. The resistance of a group to invasion and spread of an infectious agent, based on the resistance to infection of a high proportion of individual members of the group.

inapparent infection (asymptomatic infection): The presence of infection in a host without occurrence of recognizable clinical signs or symptoms. Of epidemiologic significance because hosts so infected, though apparently well, may serve as silent or inapparent disseminators of the infectious agent.

index case: the case which brings a household or other group to the attention of public health personnel.

intrinsic incubation period: The time interval between invasion by an infectious agent and appearance of the first sign or symptom of disease.

recall bias: Systematic error due to differences in accuracy or completeness of recall to memory of prior events or experiences.

reporting bias: Systematic error in the selection of information that is suppressed or revealed.

RNA virus: Virus containing ribonucleic acid genetic material.

serotype: Virus variant that produces a specific antibody reaction.

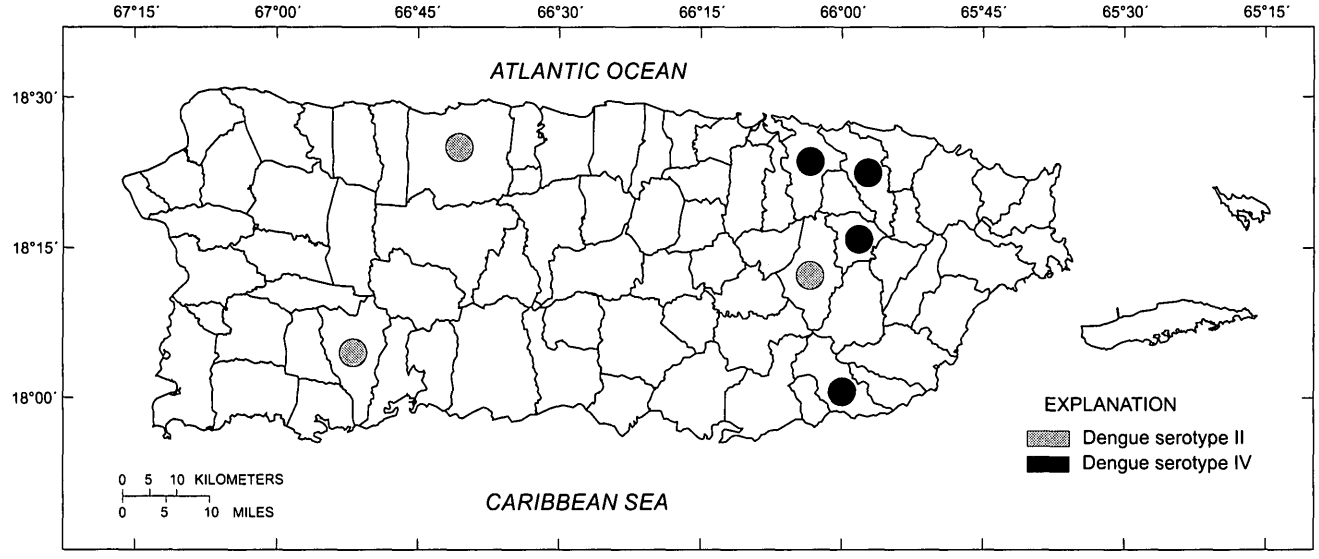
vector: An insect or any living carrier that transports an infectious agent from an infected individual to a susceptible individual. For the purposes of this report, the infectious agent (dengue virus) must pass through a developmental cycle within the vector (*Aedes aegypti*).

viremia, viremia (noun); viremic (adjective): The presence of a virus in the bloodstream.

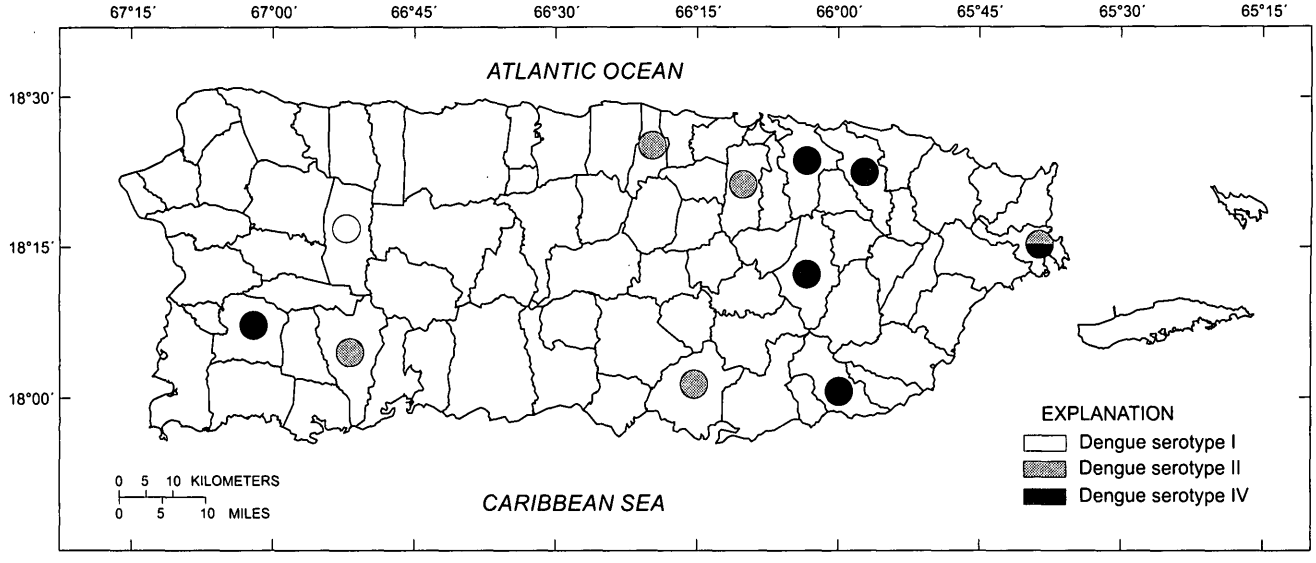
All definitions were adapted from Last (1988) and Benenson (1995).

APPENDIXES

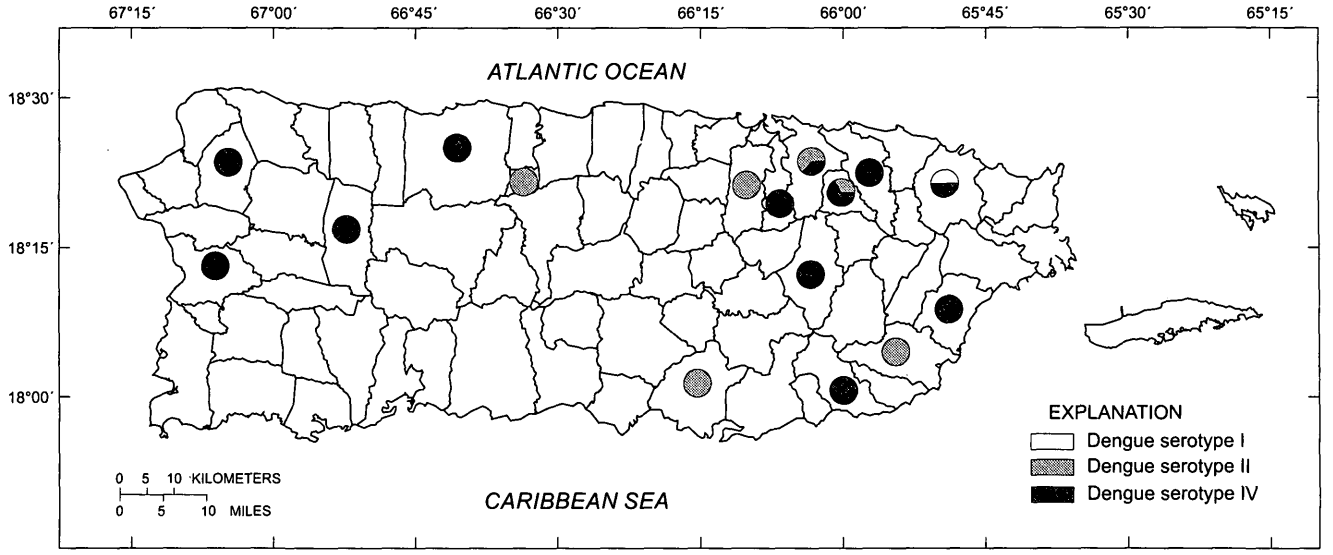
Appendix A1. Distribution of individual dengue serotype in Puerto Rico, June 1988.



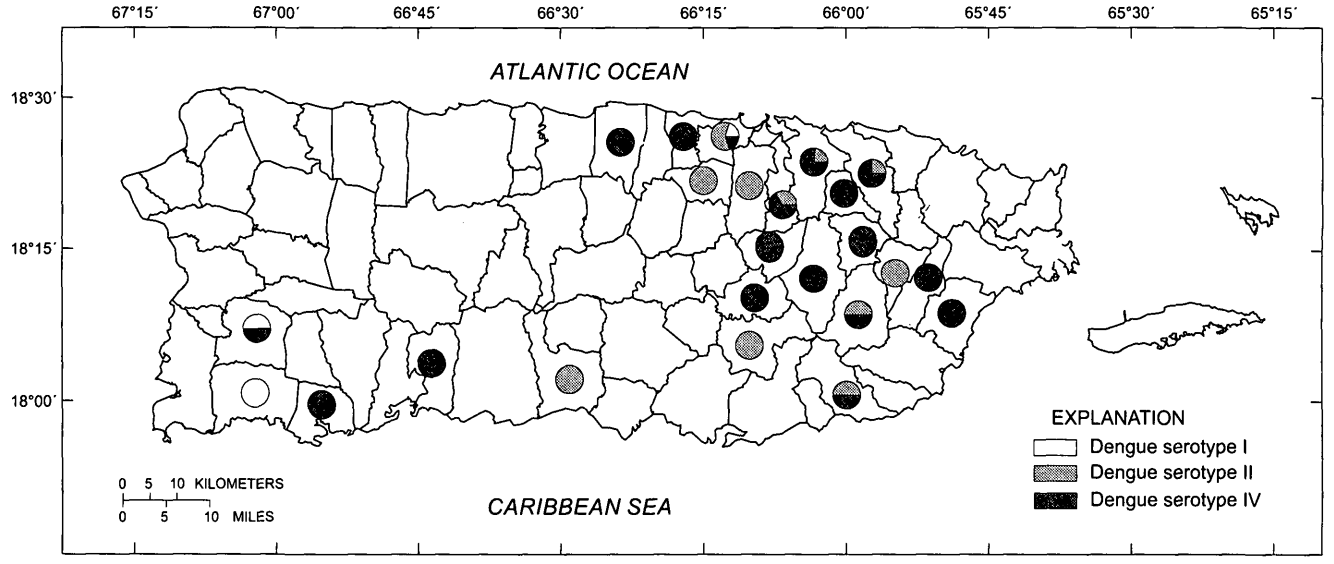
Appendix A2. Distribution of individual dengue serotype in Puerto Rico, July 1988.



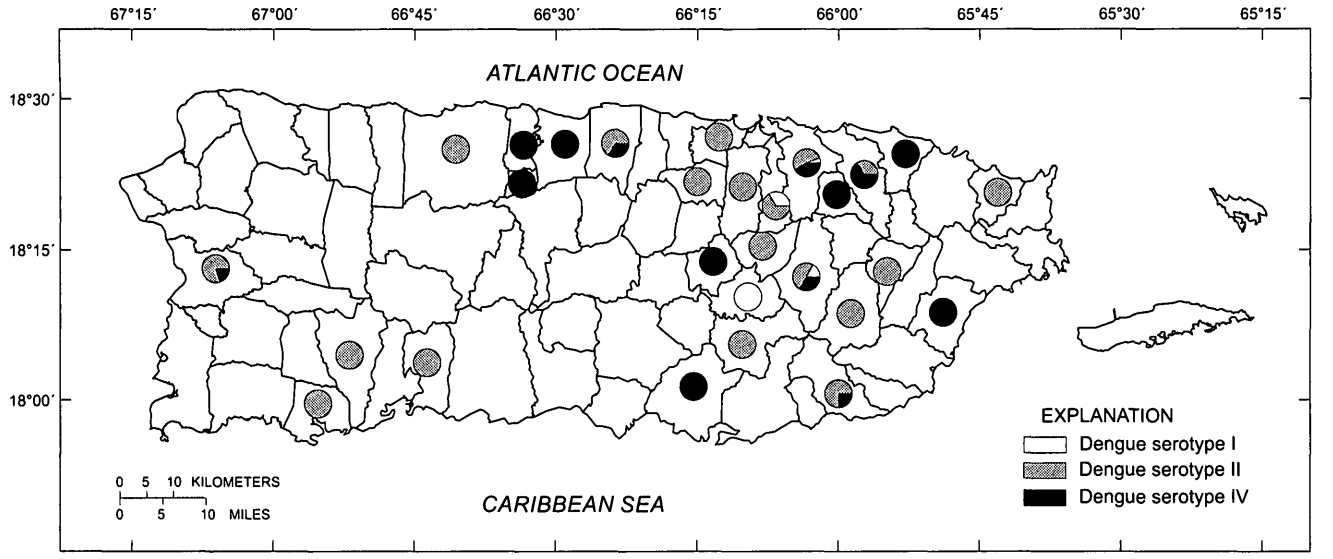
Appendix A3. Distribution of individual dengue serotype in Puerto Rico, August 1988.



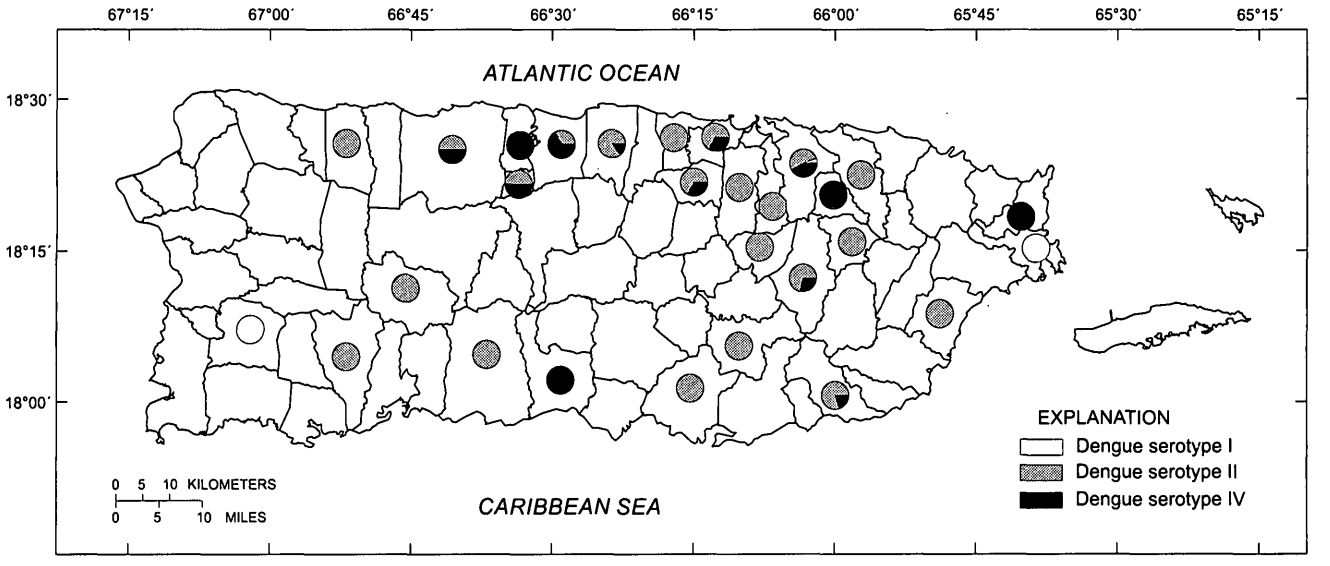
Appendix A4. Distribution of individual dengue serotype in Puerto Rico, September 1988.



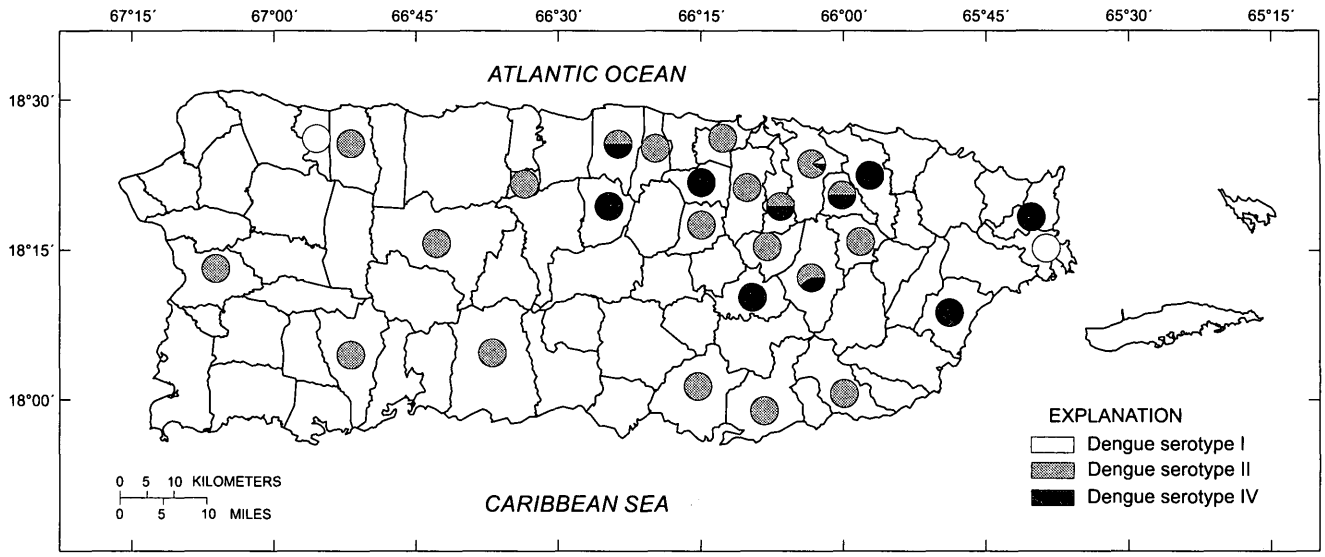
Appendix A5. Distribution of individual dengue serotype in Puerto Rico, October 1988.



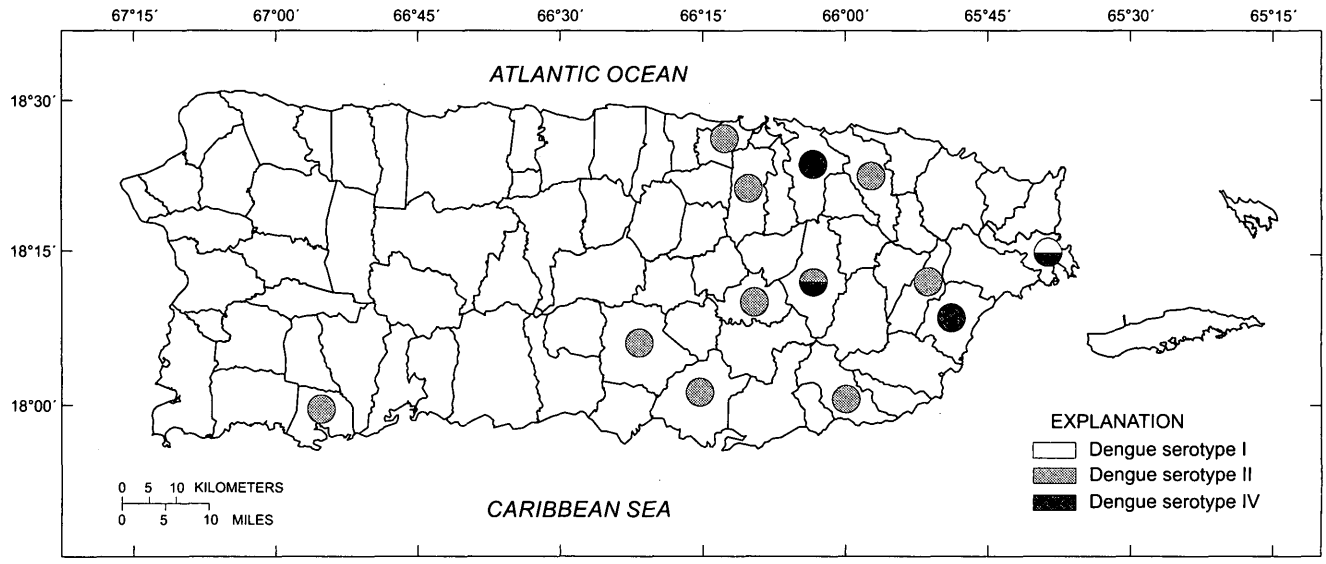
Appendix A6. Distribution of individual dengue serotype in Puerto Rico, November 1988.



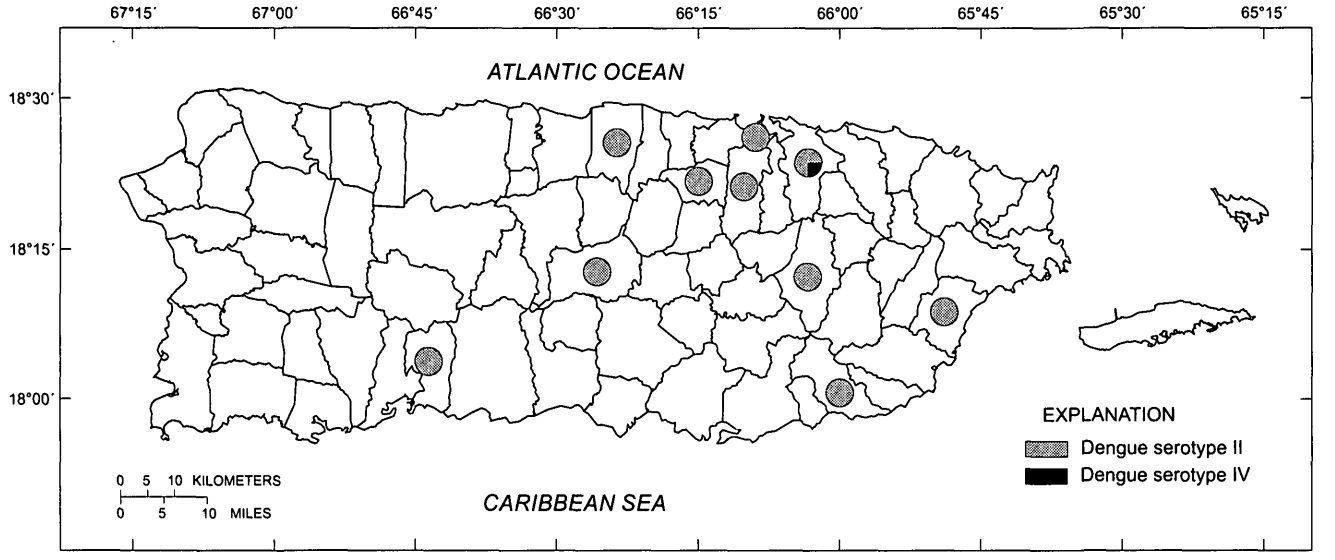
Appendix A7. Distribution of individual dengue serotype in Puerto Rico, December 1988.



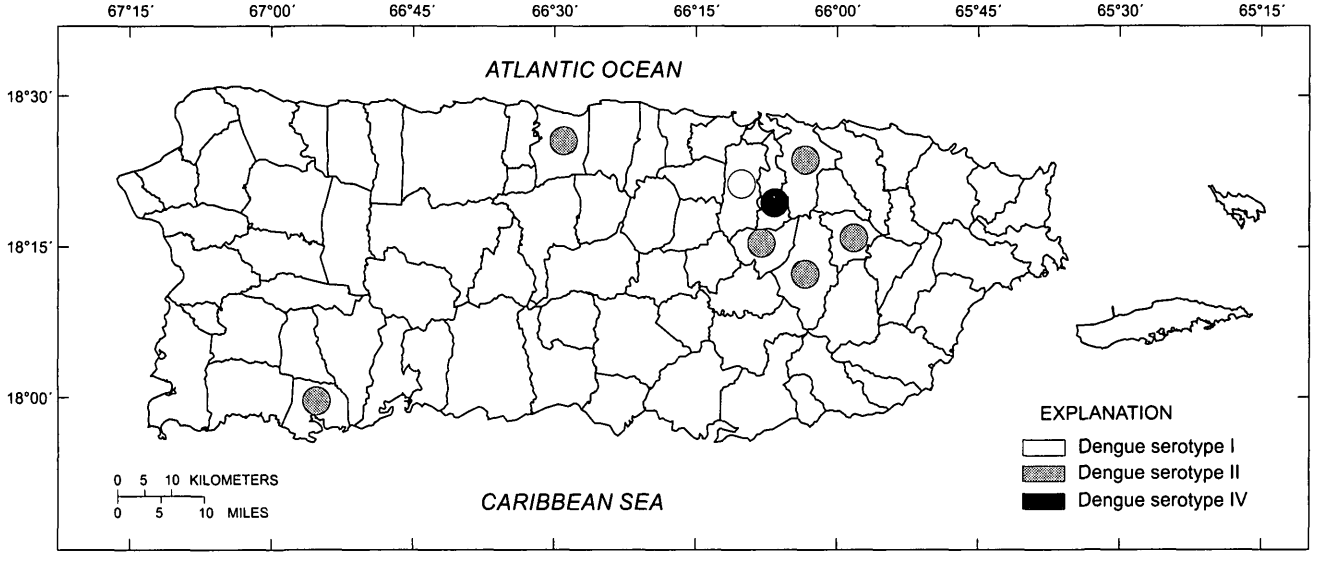
Appendix A8. Distribution of individual dengue serotype in Puerto Rico, January 1989.



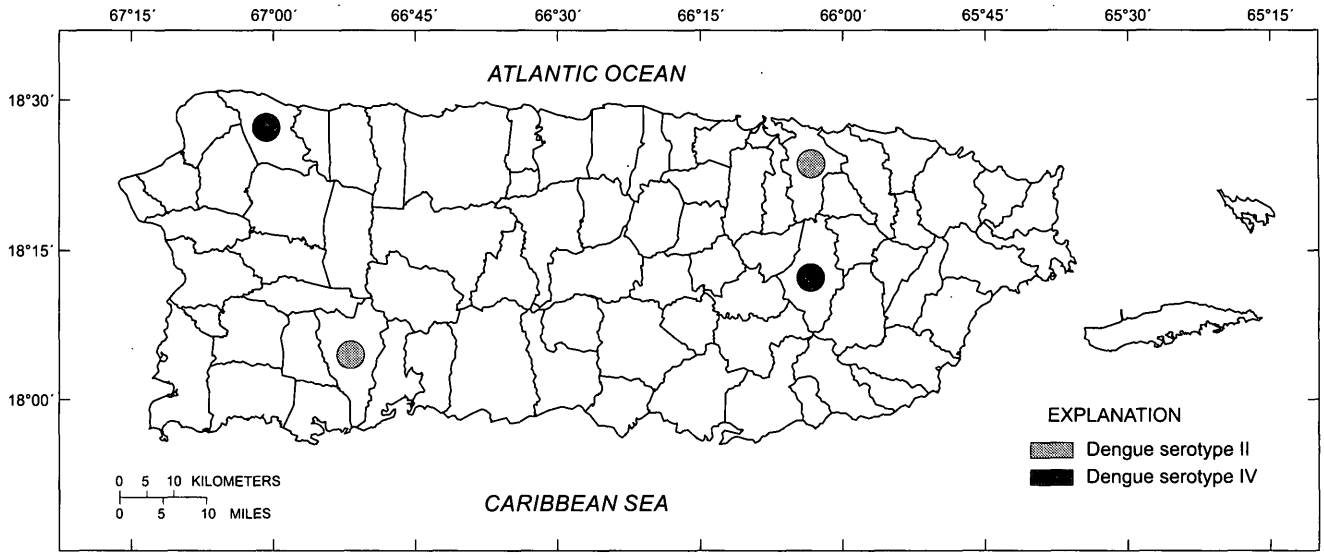
Appendix A9. Distribution of individual dengue serotype in Puerto Rico, February 1989.



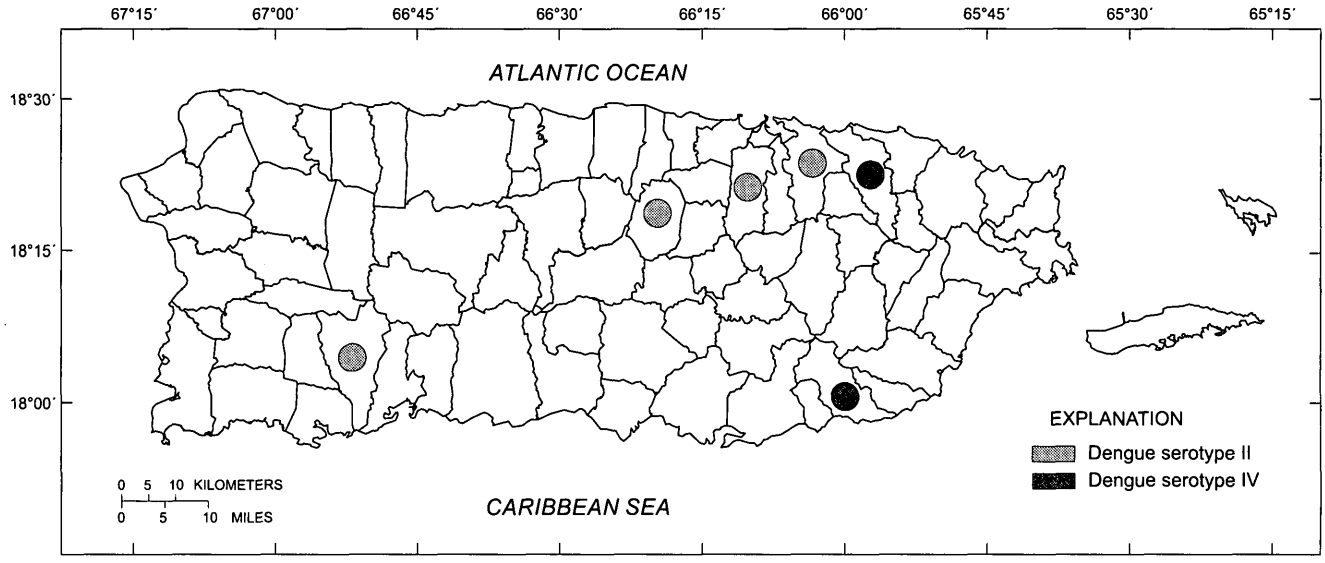
Appendix A10. Distribution of individual dengue serotype in Puerto Rico, March 1989.



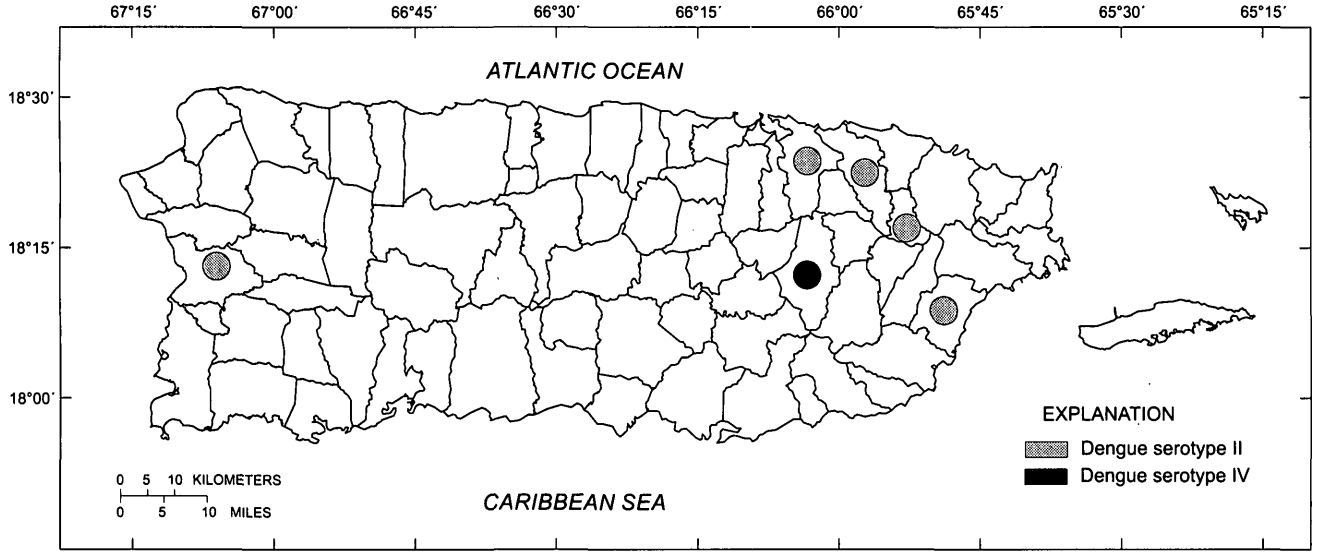
Appendix A11. Distribution of individual dengue serotype in Puerto Rico, April 1989.



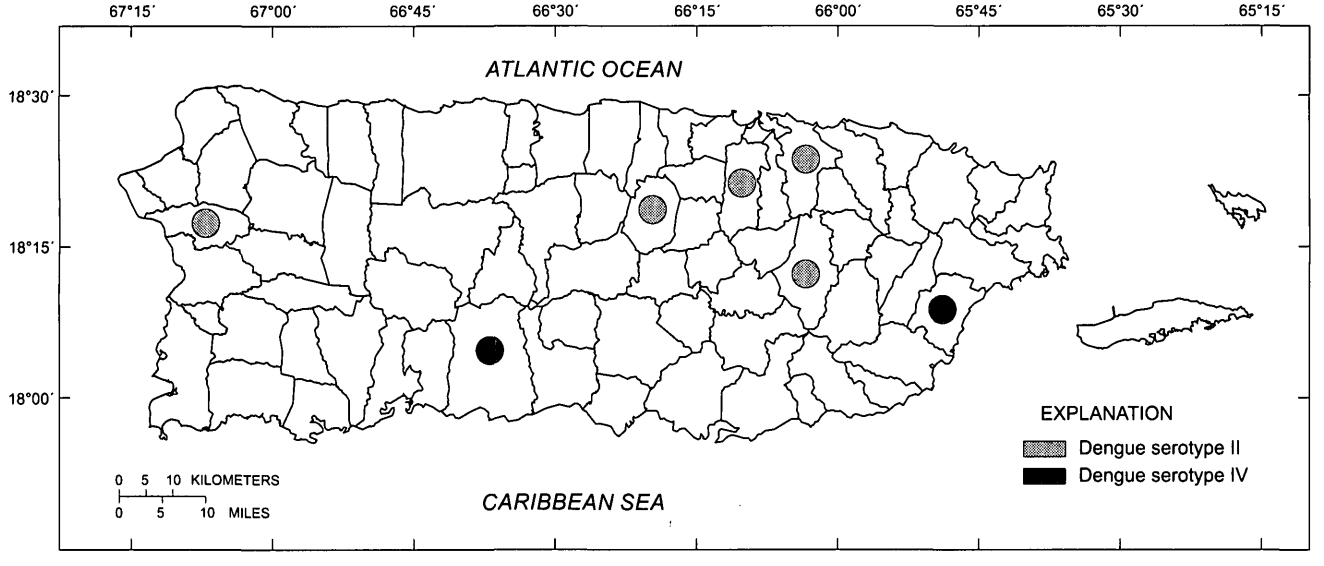
Appendix A12. Distribution of individual dengue serotype in Puerto Rico, May 1989.



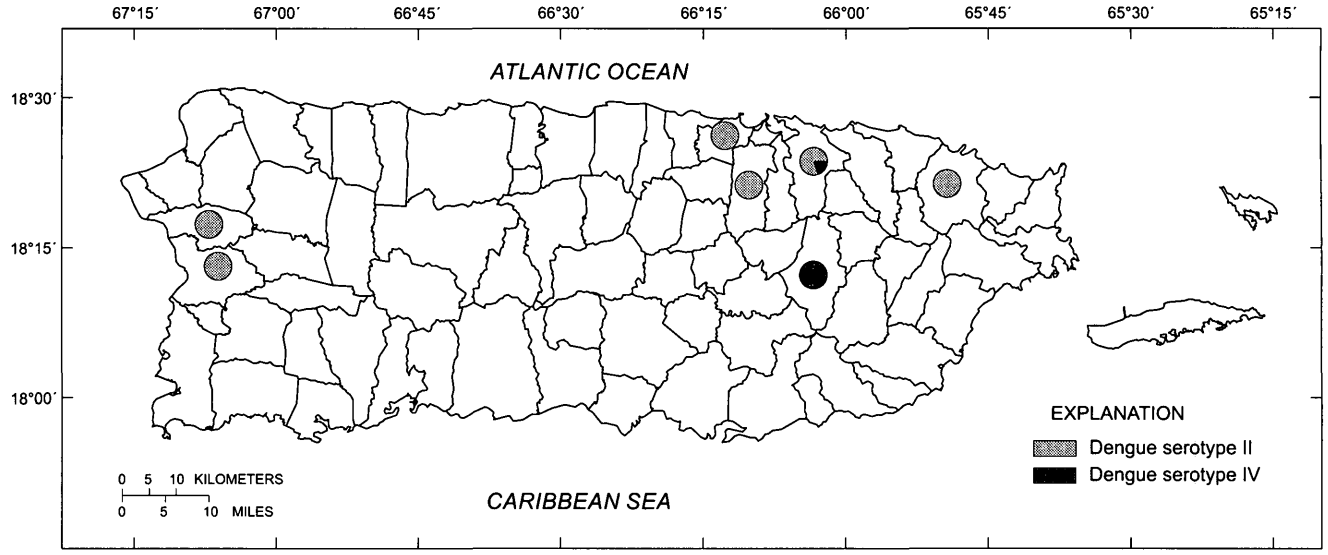
Appendix B1. Distribution of individual dengue serotype in Puerto Rico, June 1989.



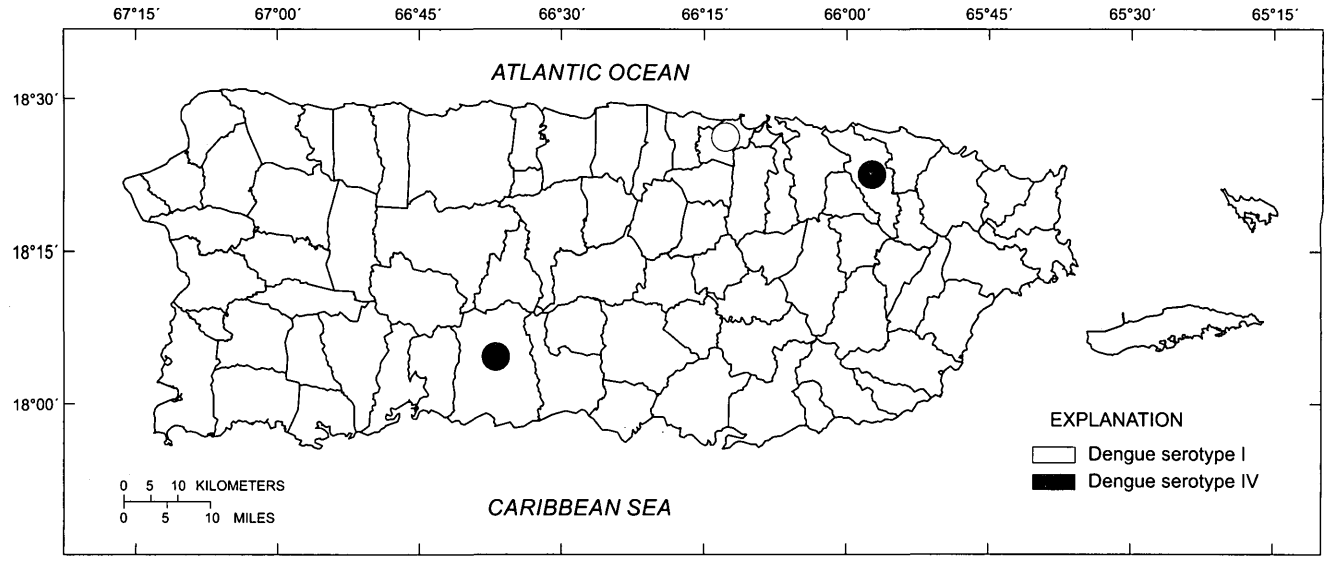
Appendix B2. Distribution of individual dengue serotype in Puerto Rico, July 1989.



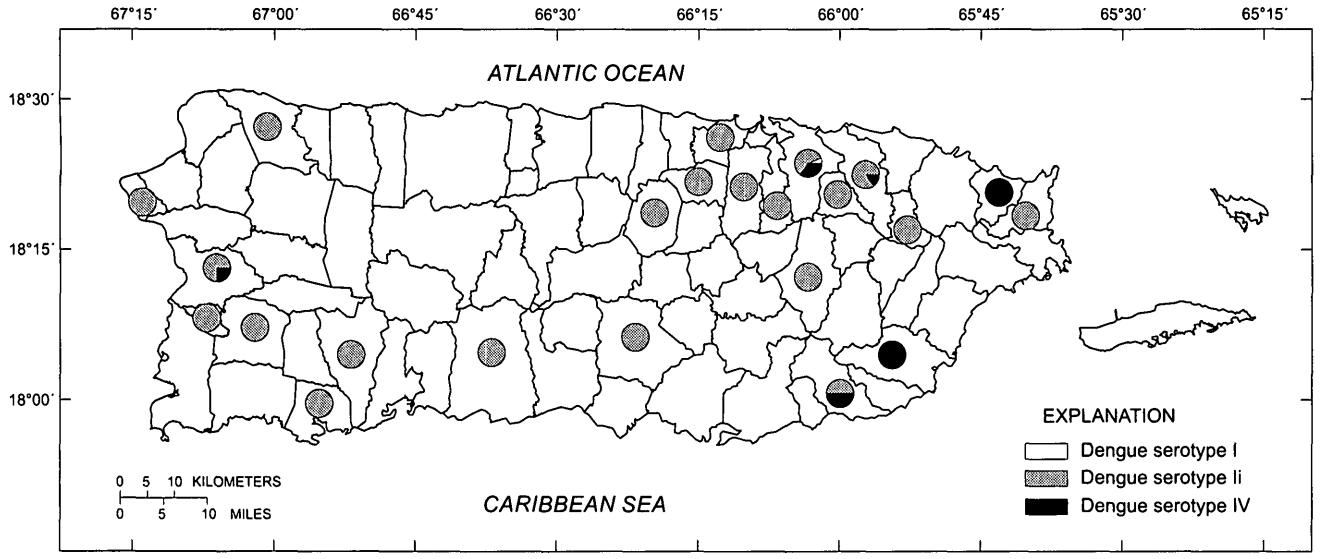
Appendix B3. Distribution of individual dengue serotype in Puerto Rico, August 1989.



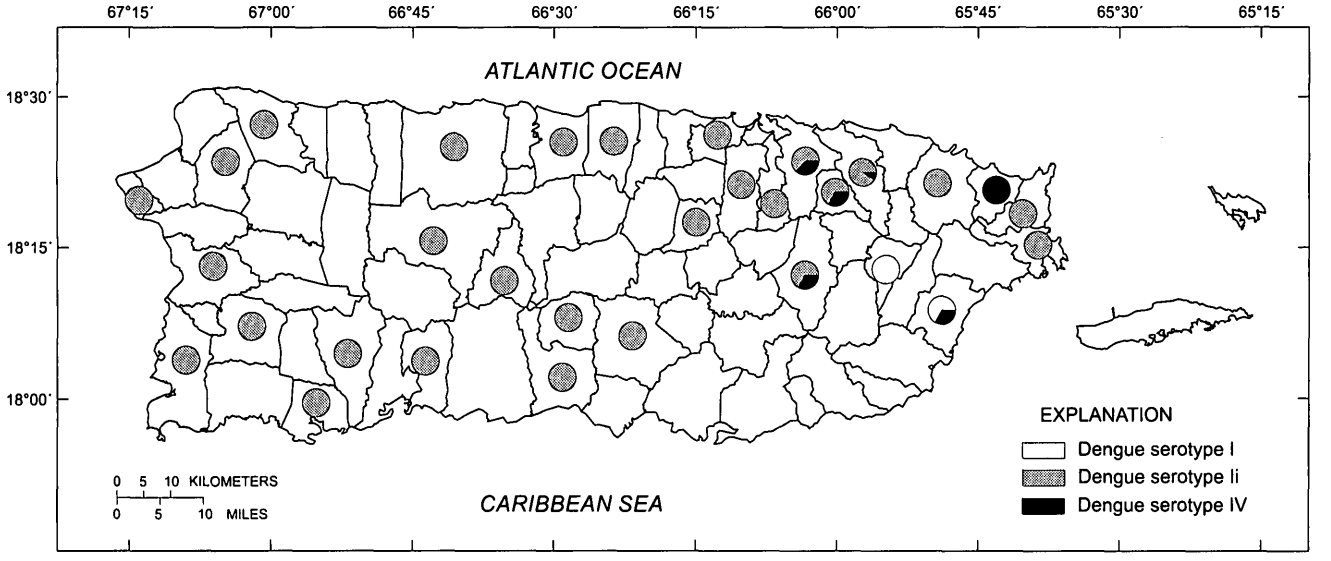
Appendix B4. Distribution of individual dengue serotype in Puerto Rico, September 1989.



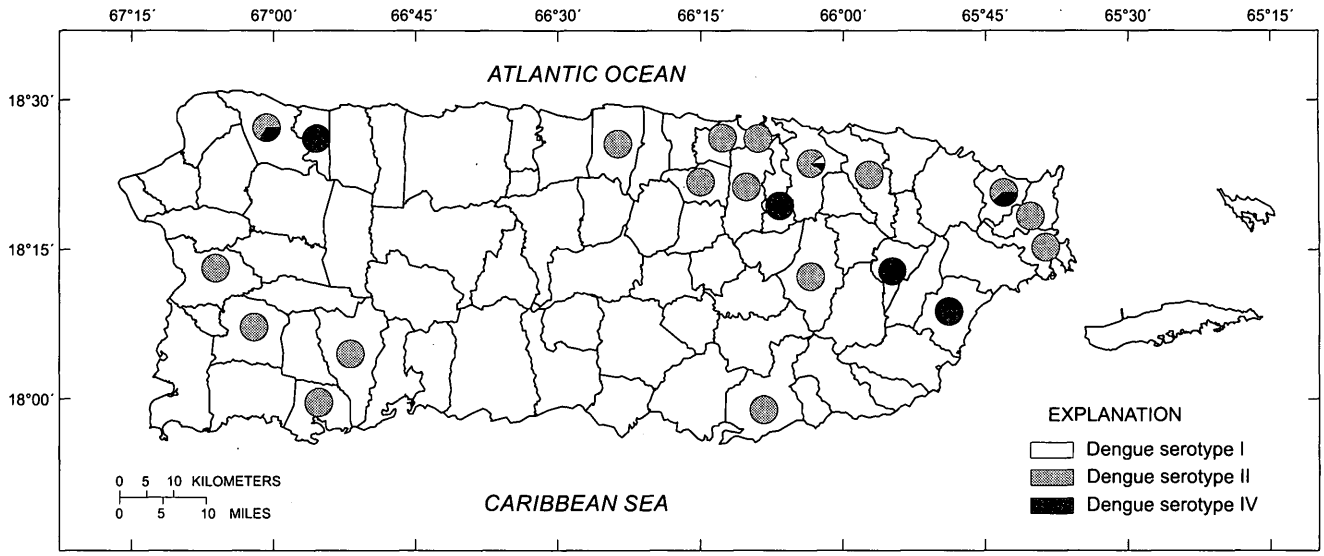
Appendix B5. Distribution of individual dengue serotype in Puerto Rico, October 1989.



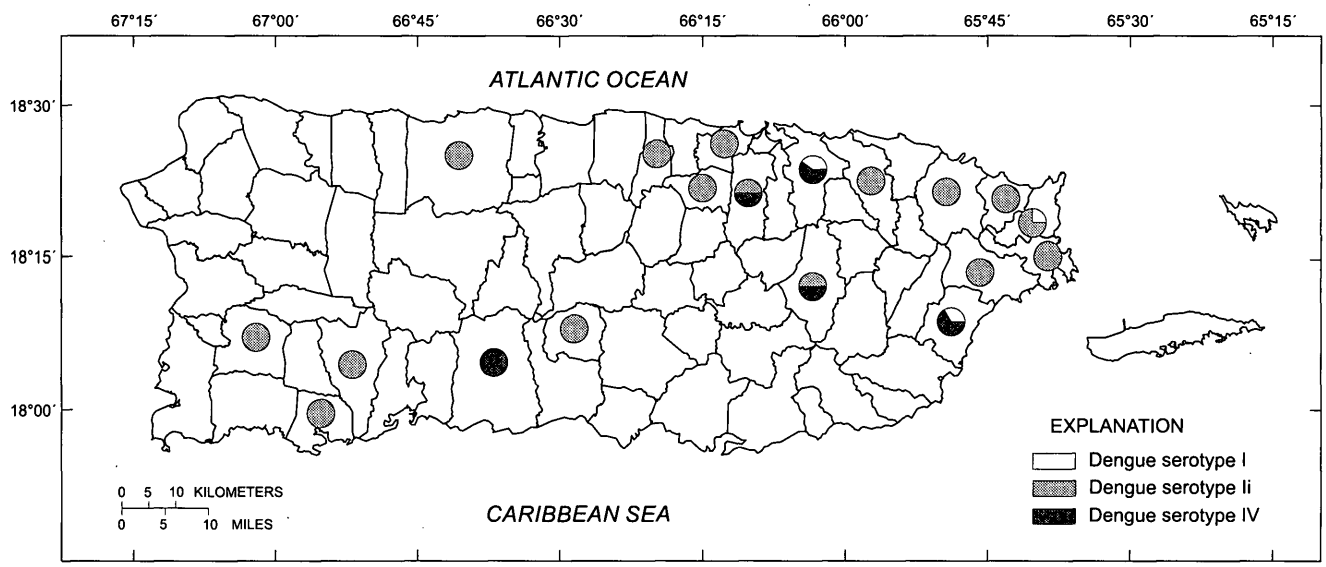
Appendix B6. Distribution of individual dengue serotype in Puerto Rico, November 1989.



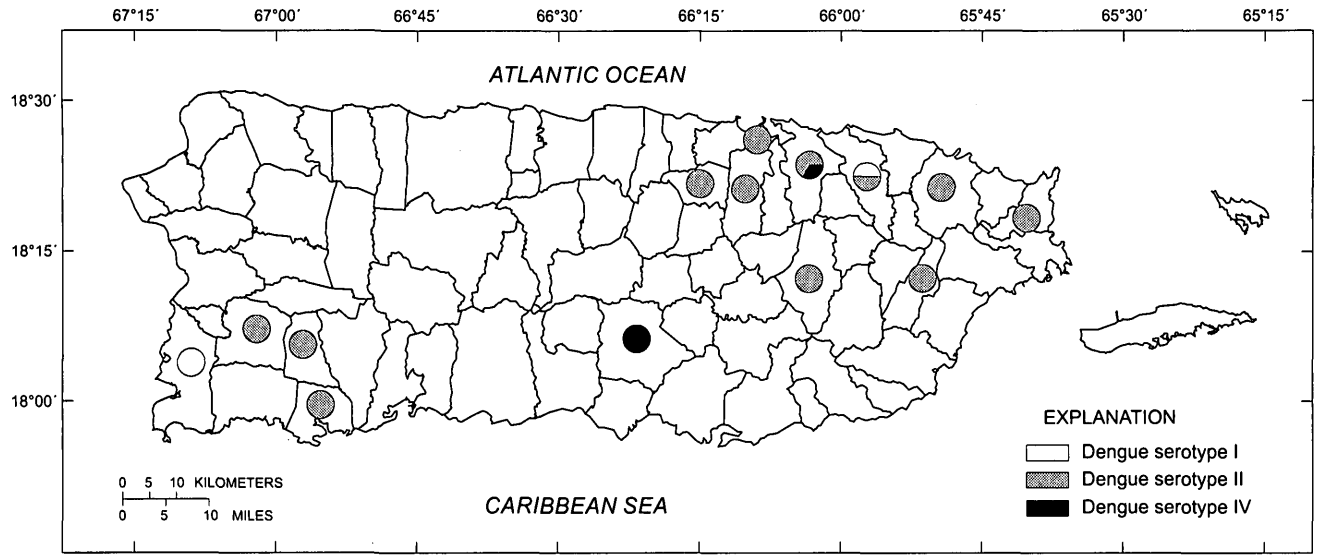
Appendix B7. Distribution of individual dengue serotype in Puerto Rico, December 1989.



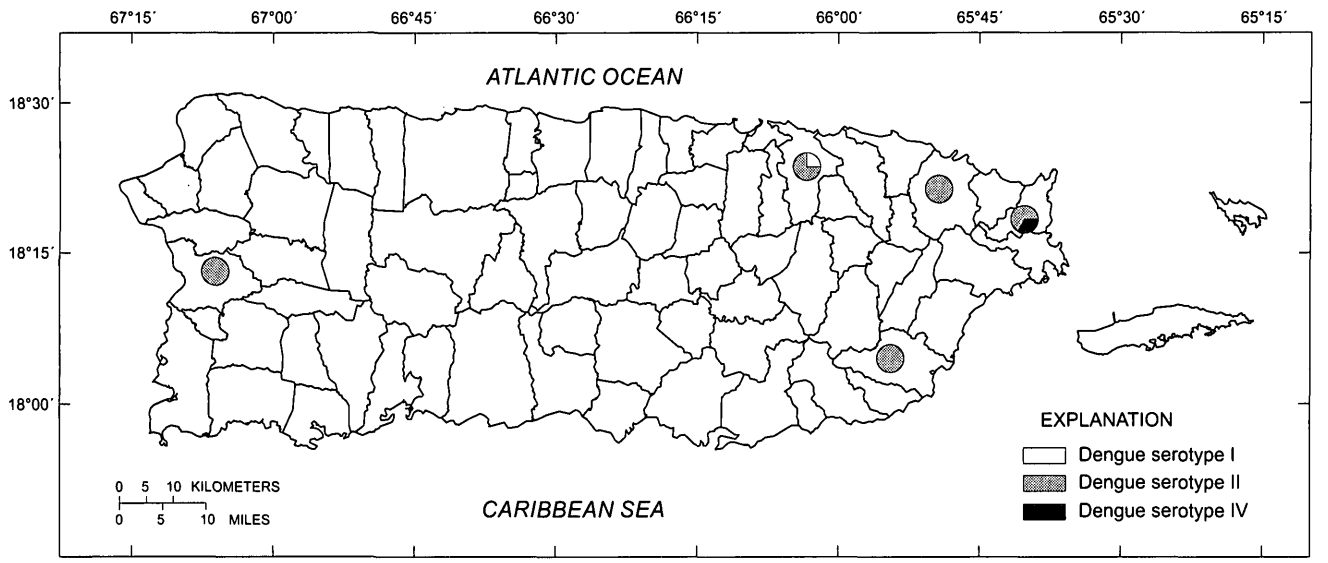
Appendix B8. Distribution of individual dengue serotype in Puerto Rico, January 1990.



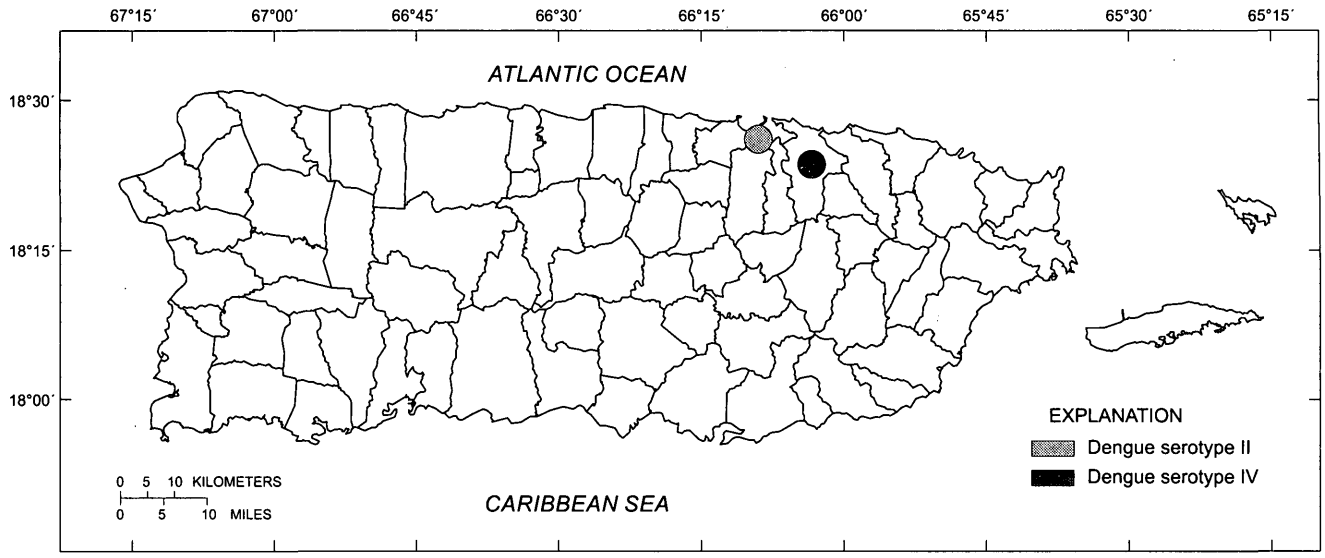
Appendix B9. Distribution of individual dengue serotype in Puerto Rico, February 1990.



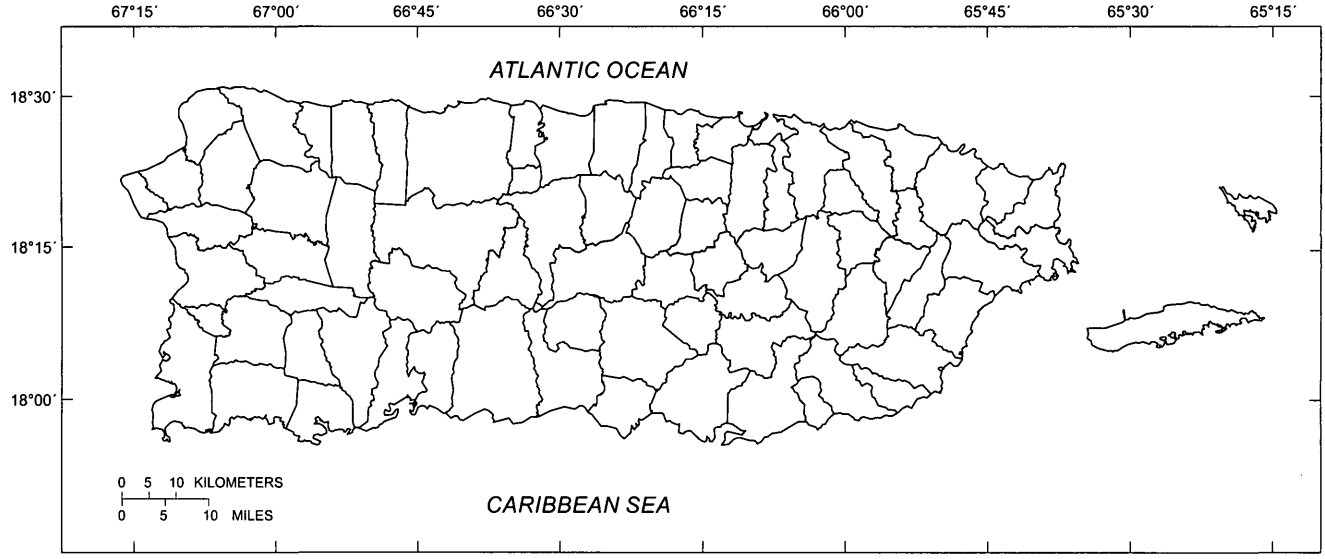
Appendix B10. Distribution of individual dengue serotype in Puerto Rico, March 1990.



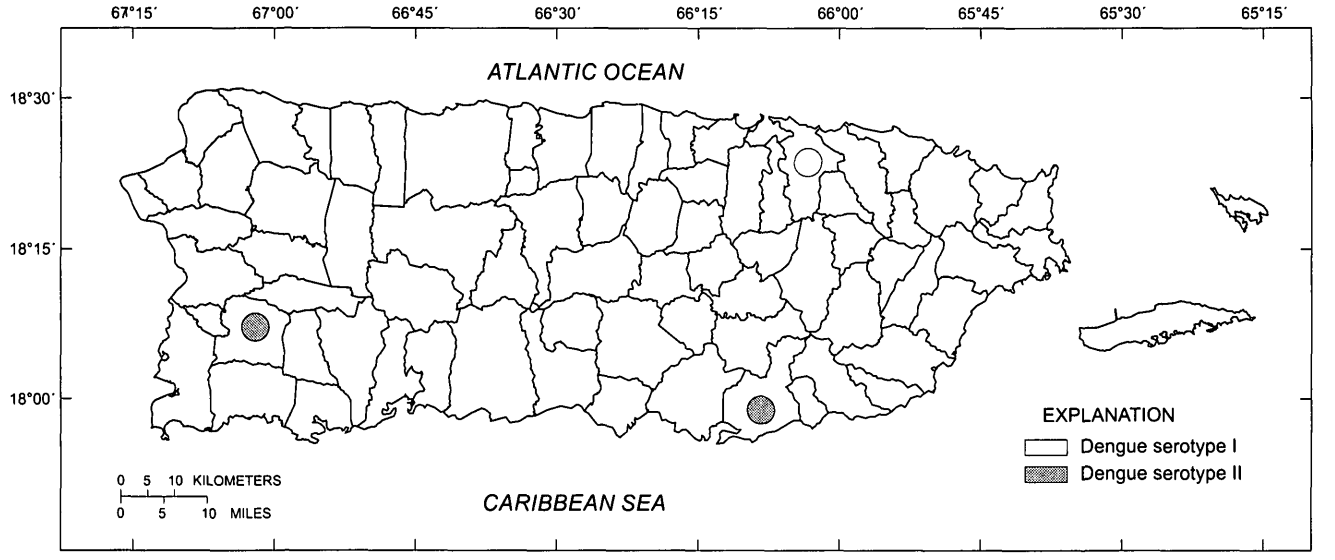
Appendix B11. Distribution of individual dengue serotype in Puerto Rico, April 1990.



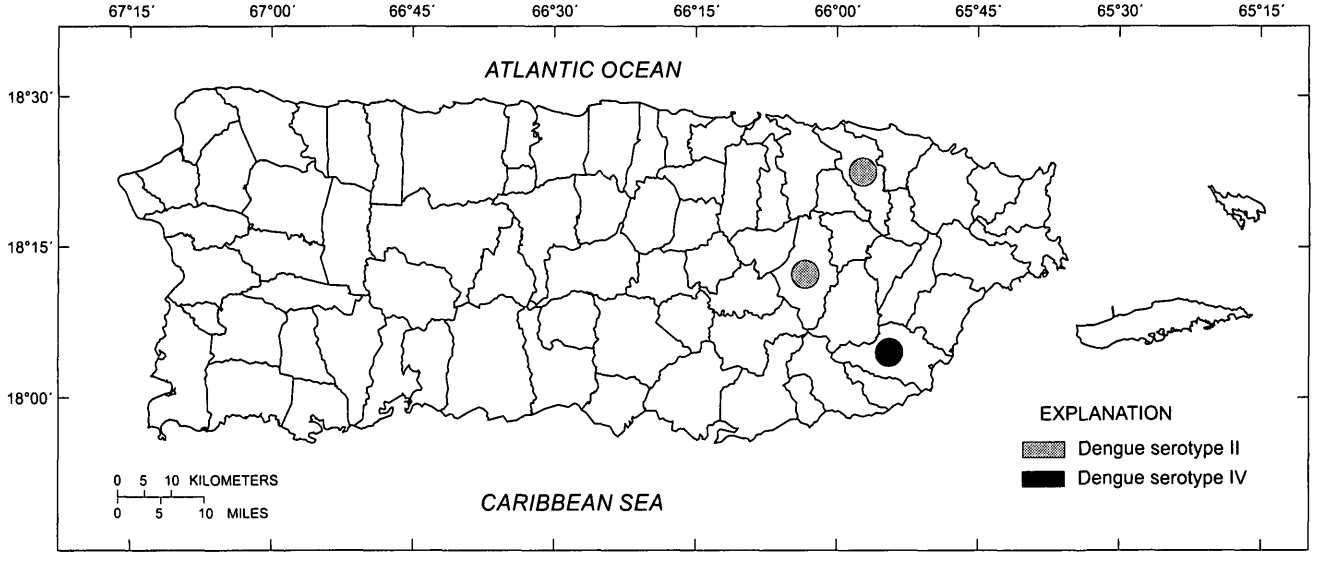
Appendix B12. Distribution of individual dengue serotype in Puerto Rico, May 1990.



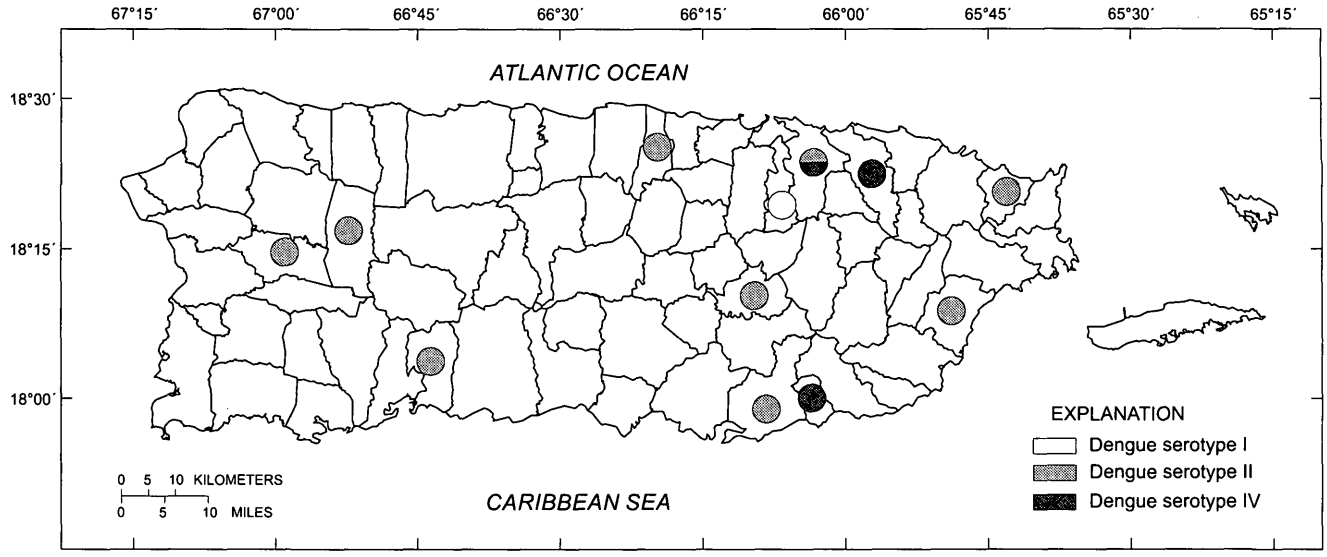
Appendix C1. Distribution of individual dengue serotype in Puerto Rico, June 1990.



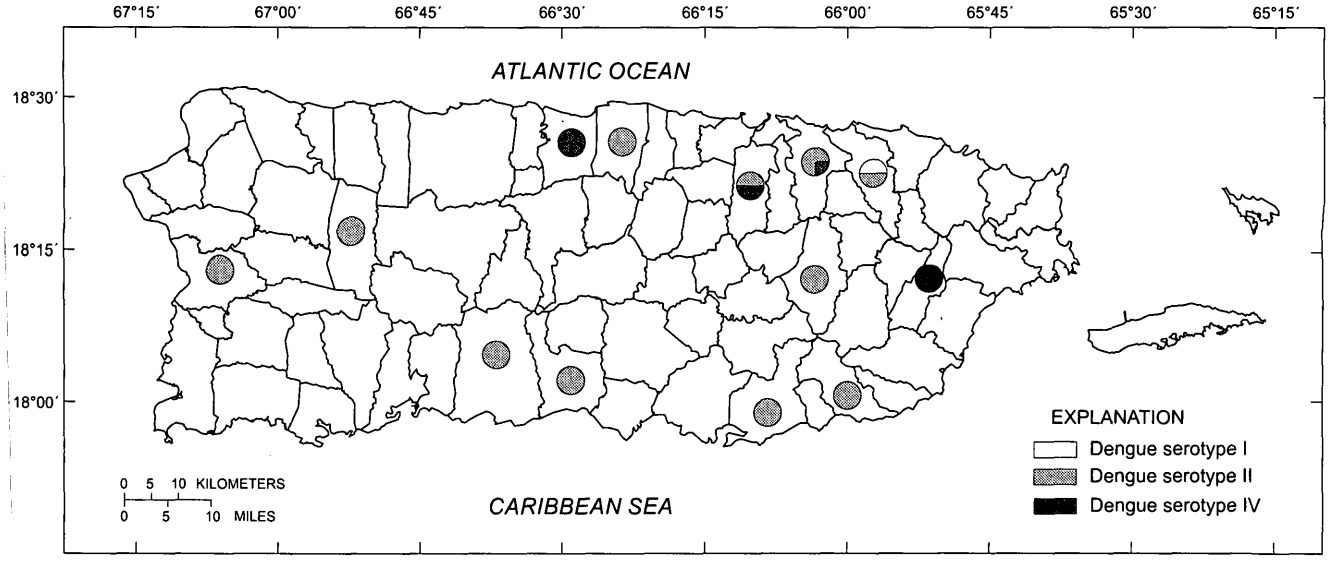
Appendix C2. Distribution of individual dengue serotype in Puerto Rico, July 1990.



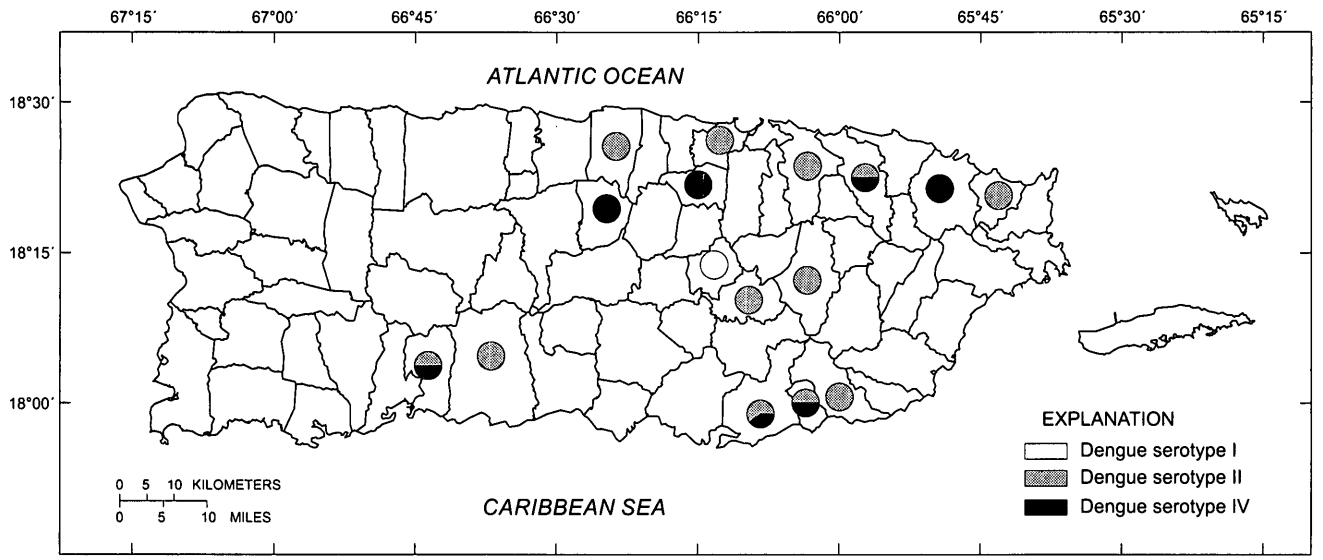
Appendix C3. Distribution of individual dengue serotype in Puerto Rico, August 1990.



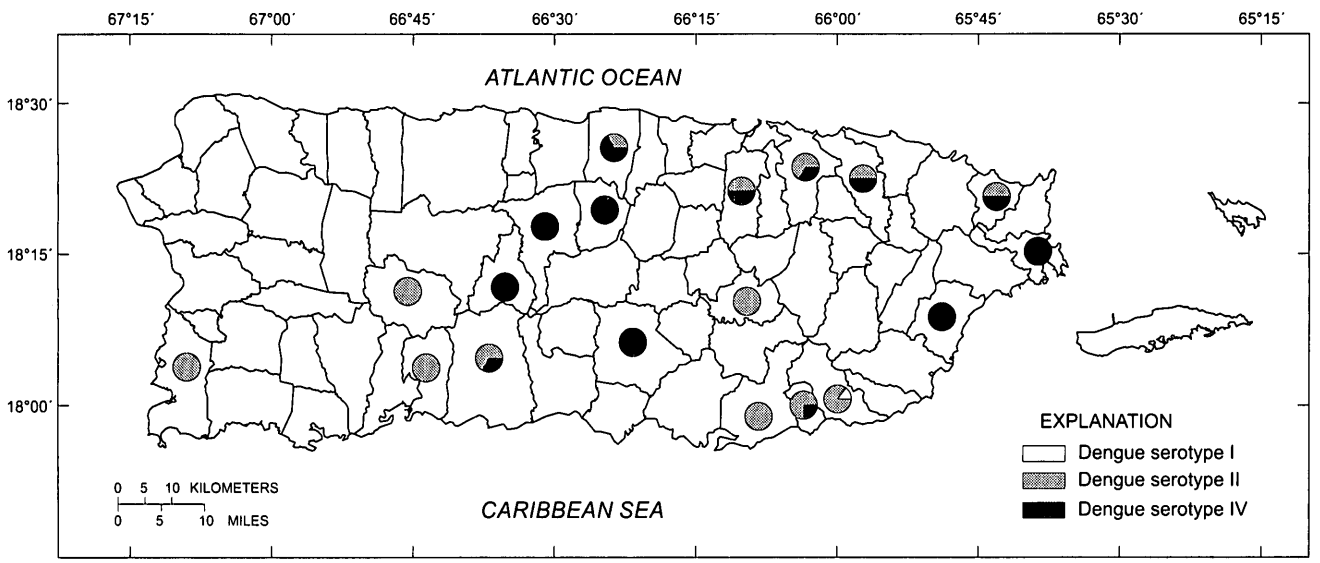
Appendix C4. Distribution of individual dengue serotype in Puerto Rico, September 1990.



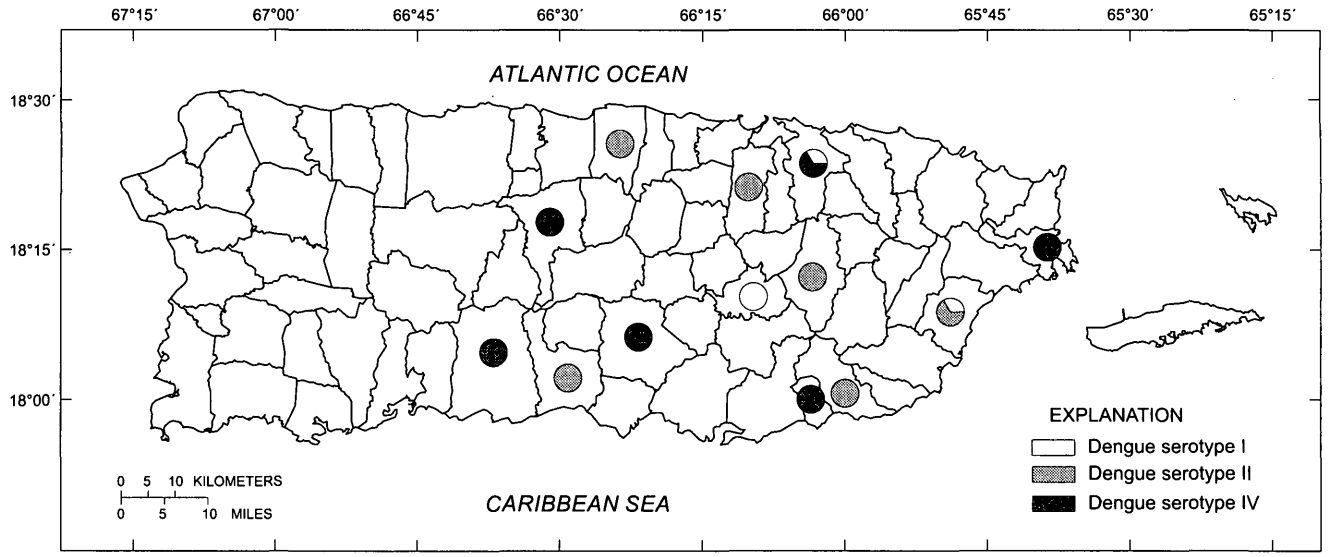
Appendix C5. Distribution of individual dengue serotype in Puerto Rico, October 1990.



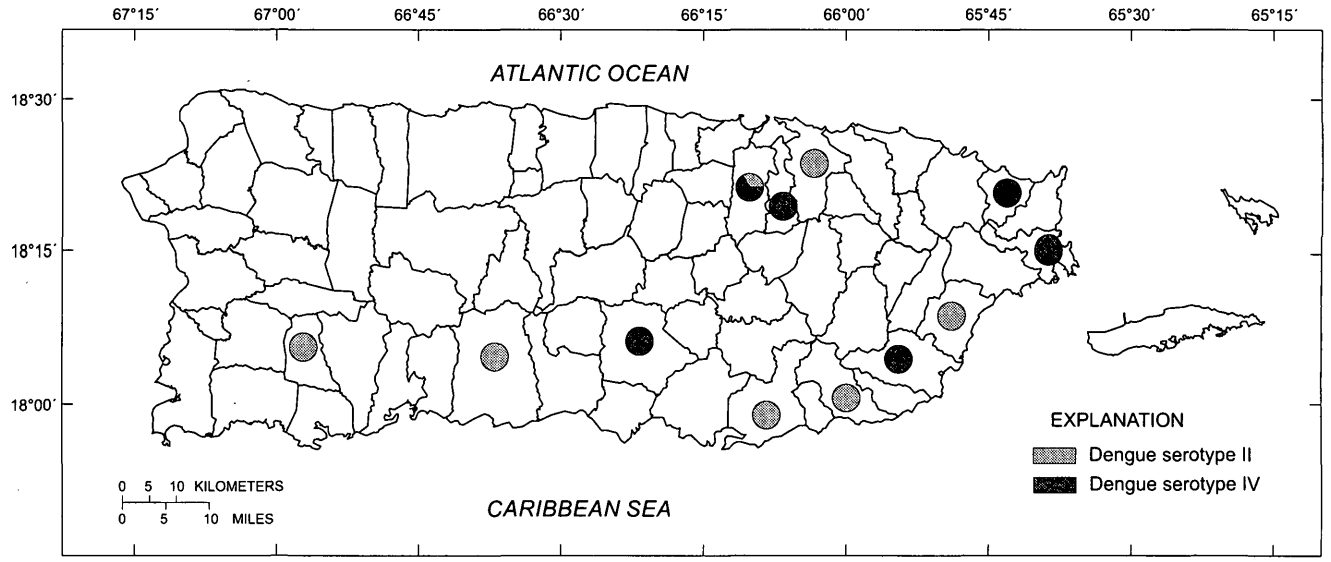
Appendix C6. Distribution of individual dengue serotype in Puerto Rico, November 1990.



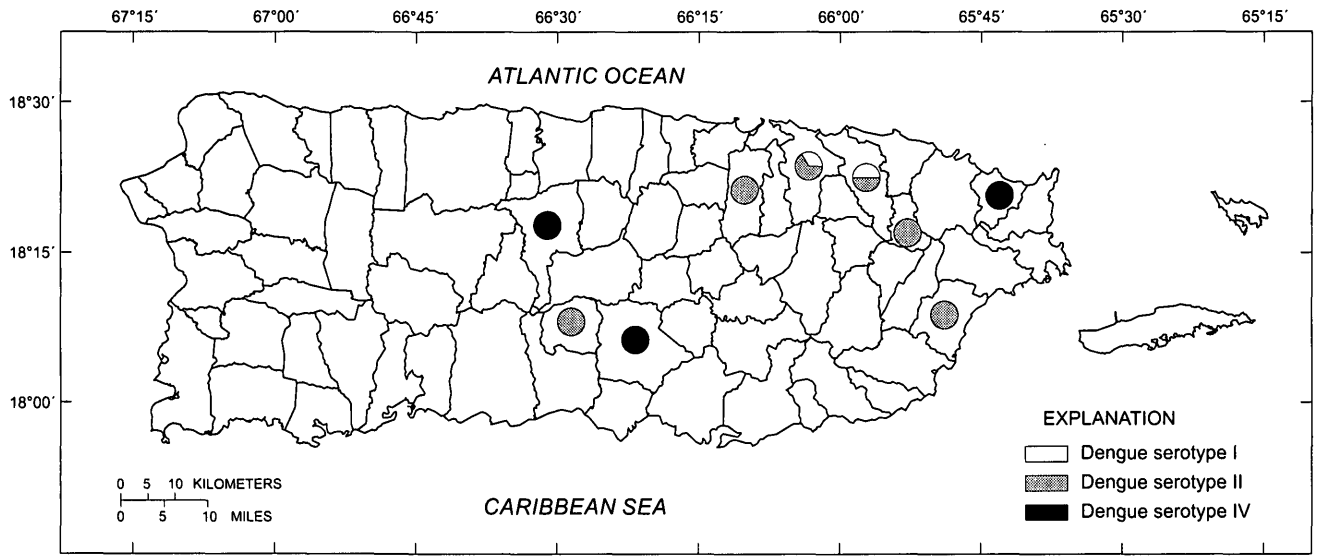
Appendix C7. Distribution of individual dengue serotype in Puerto Rico, December 1990.



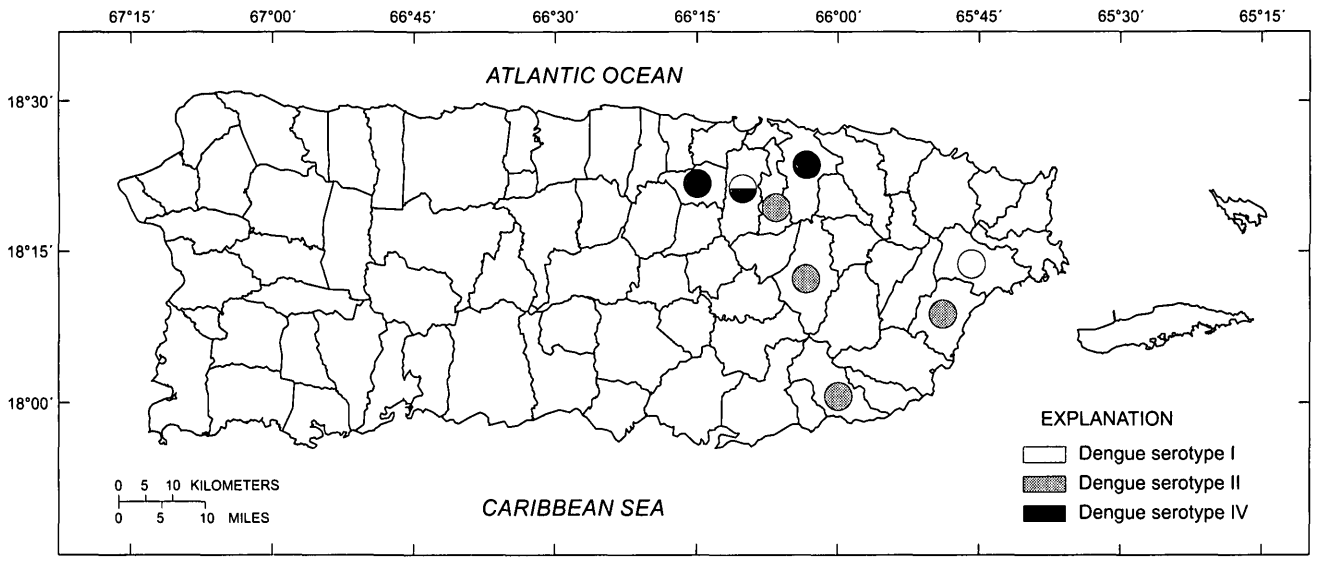
Appendix C8. Distribution of individual dengue serotype in Puerto Rico, January 1991.



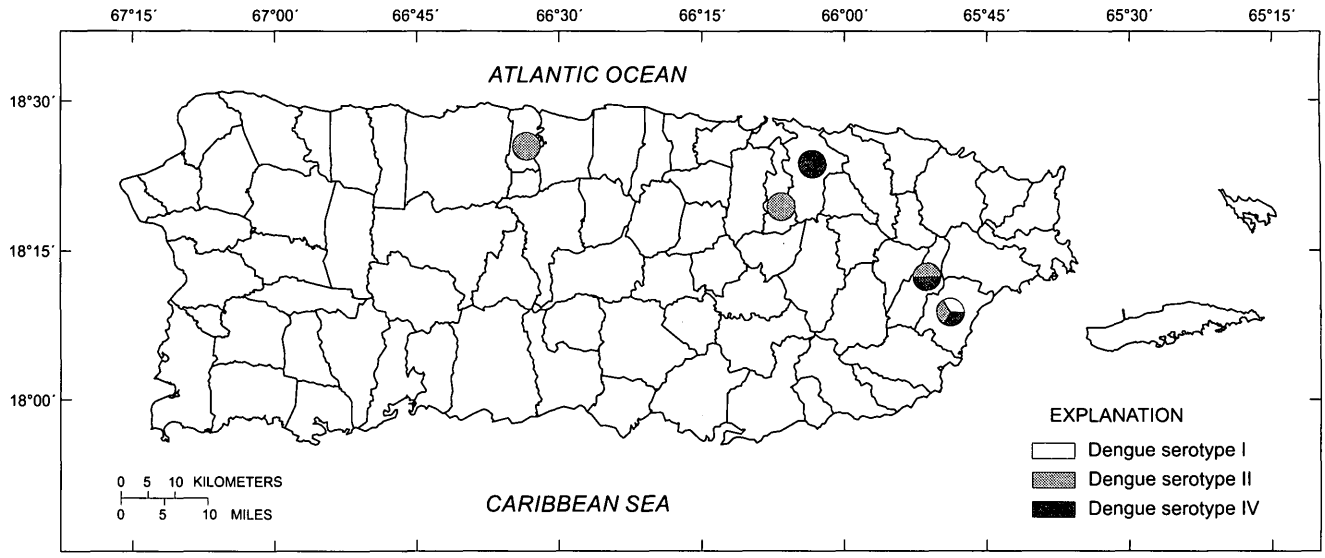
Appendix C9. Distribution of individual dengue serotype in Puerto Rico, February 1991.



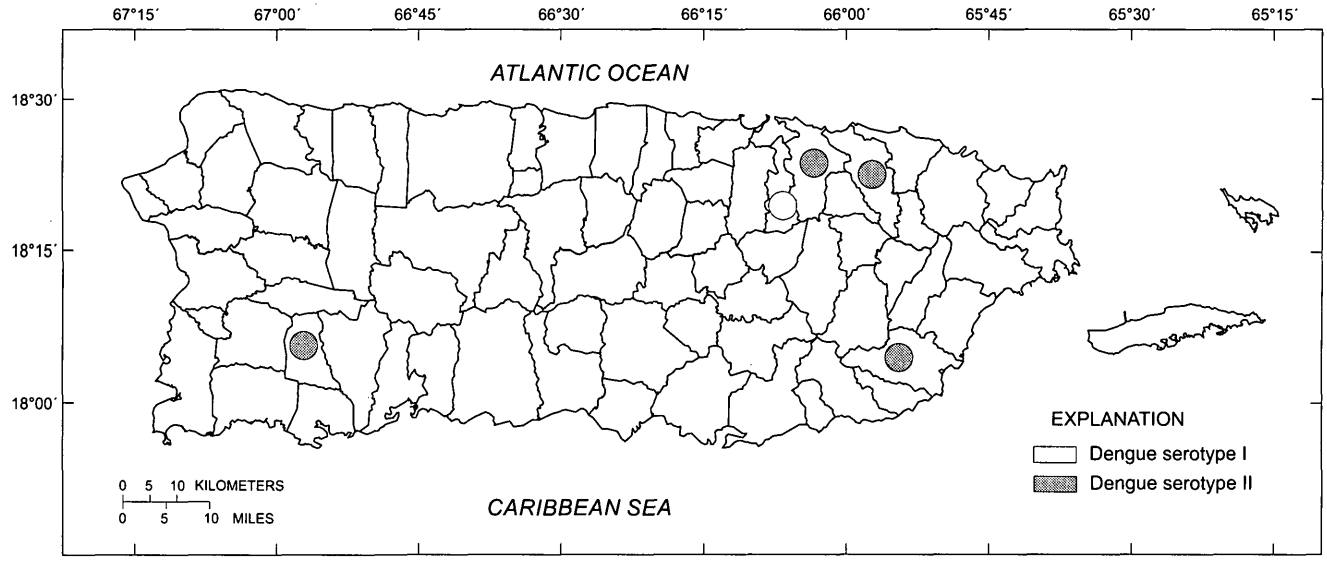
Appendix C10. Distribution of individual dengue serotype in Puerto Rico, March 1991.



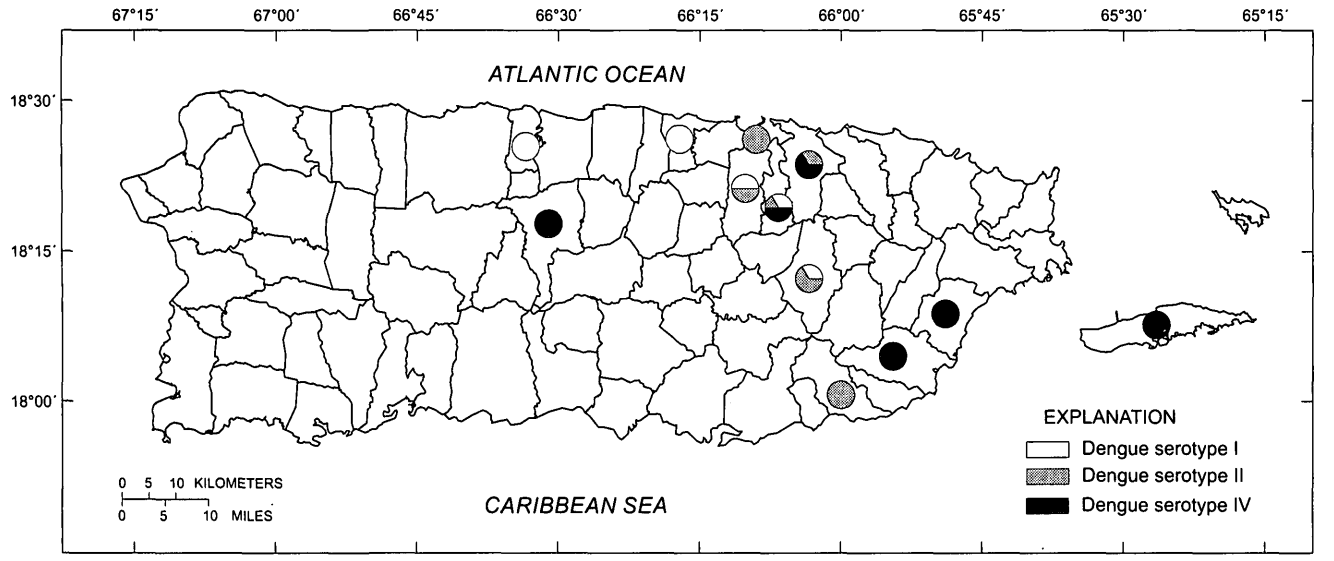
Appendix C11. Distribution of individual dengue serotype in Puerto Rico, April 1991.



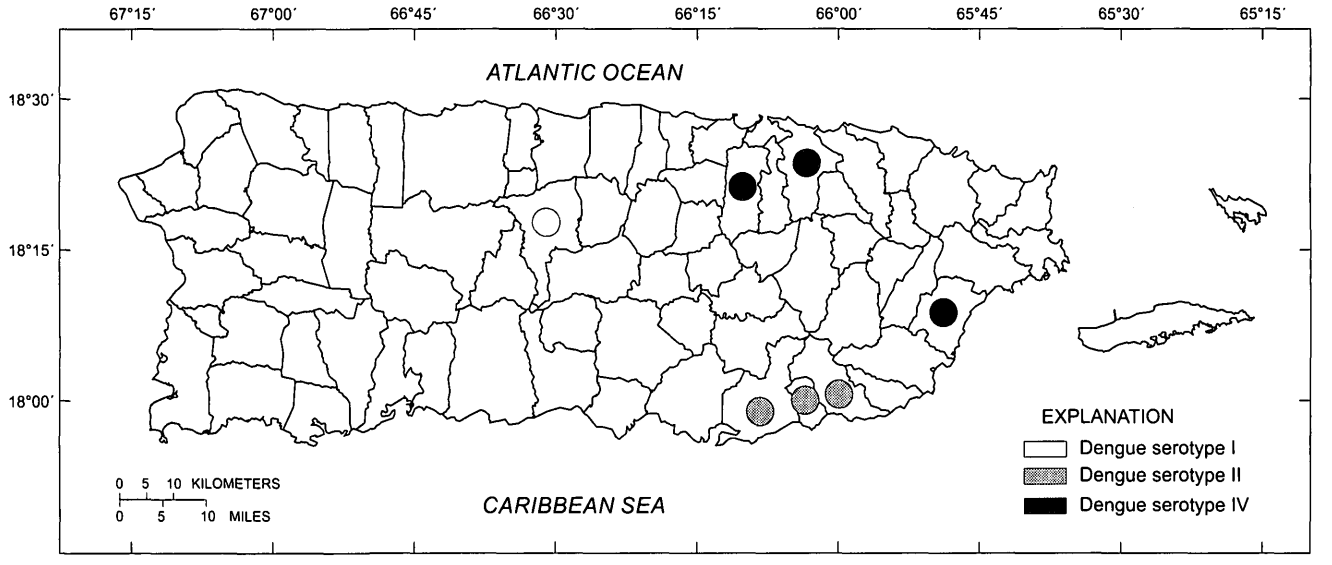
Appendix C12. Distribution of individual dengue serotype in Puerto Rico, May 1991.



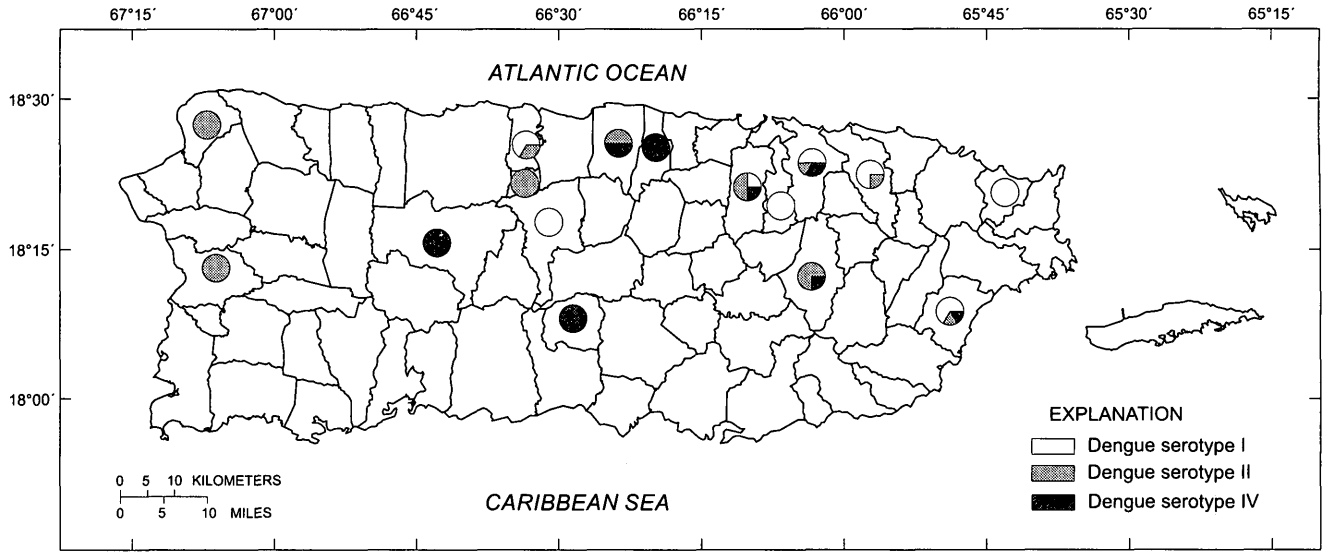
Appendix D1. Distribution of individual dengue serotype in Puerto Rico, June 1991.



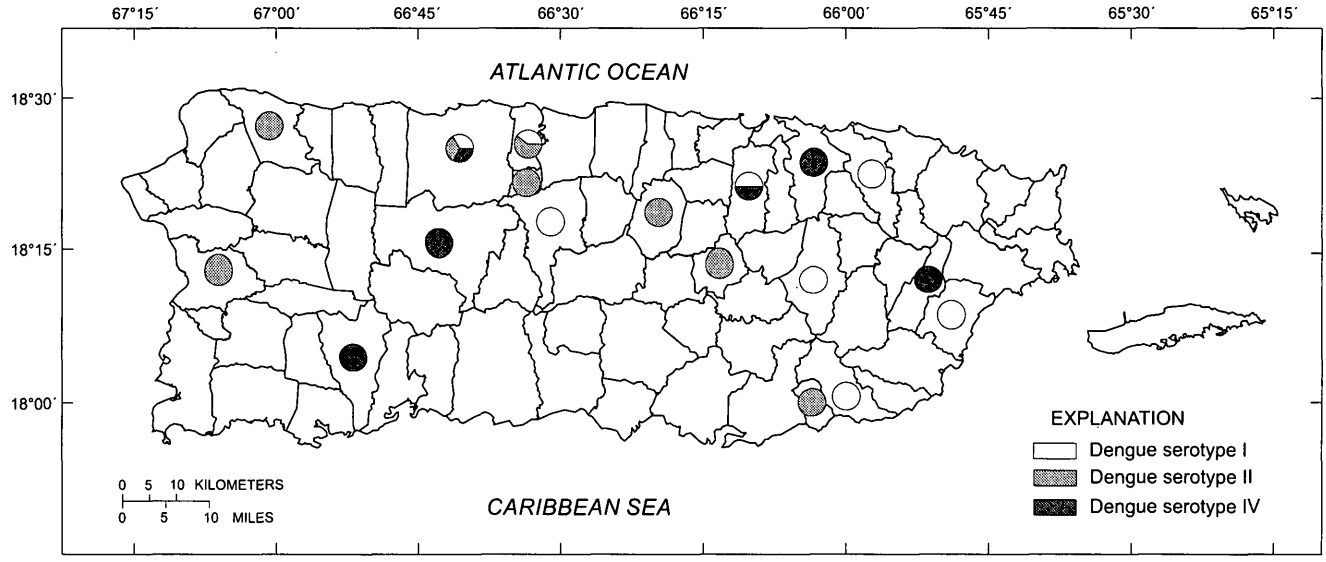
Appendix D2. Distribution of individual dengue serotype in Puerto Rico, July 1991.



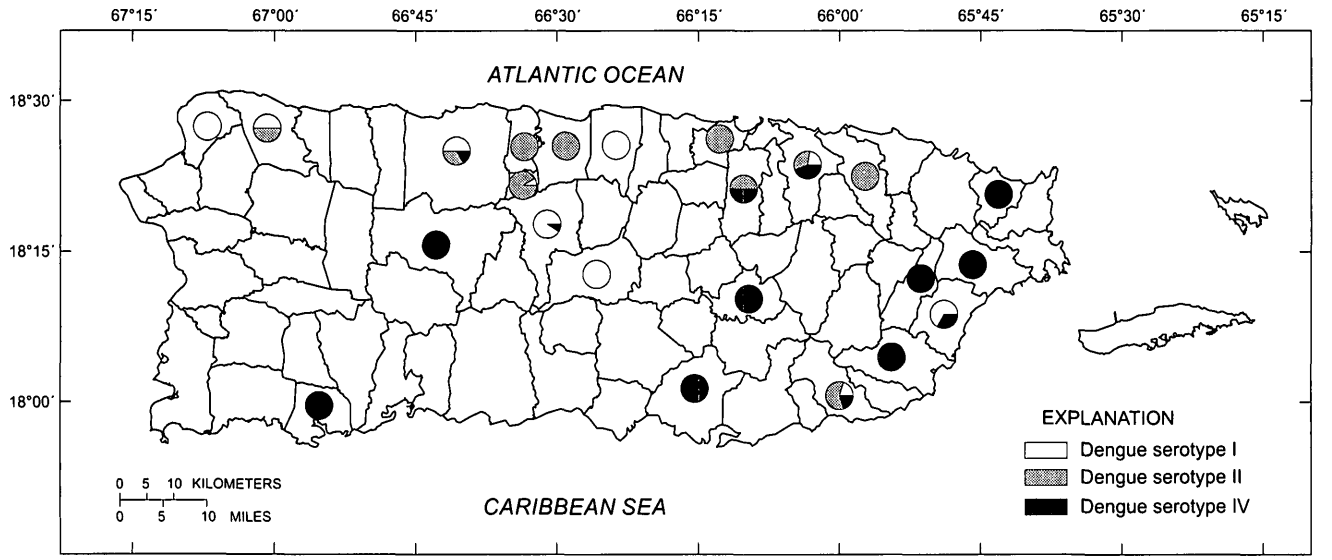
Appendix D3. Distribution of individual dengue serotype in Puerto Rico, August 1991.



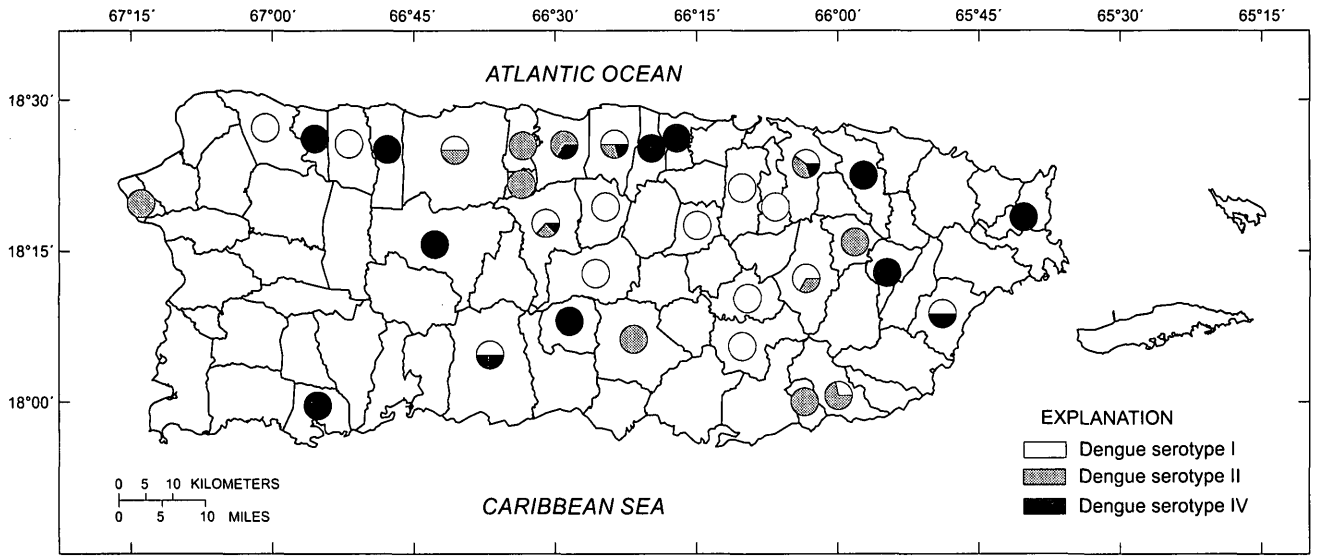
Appendix D4. Distribution of individual dengue serotype in Puerto Rico, September 1991.



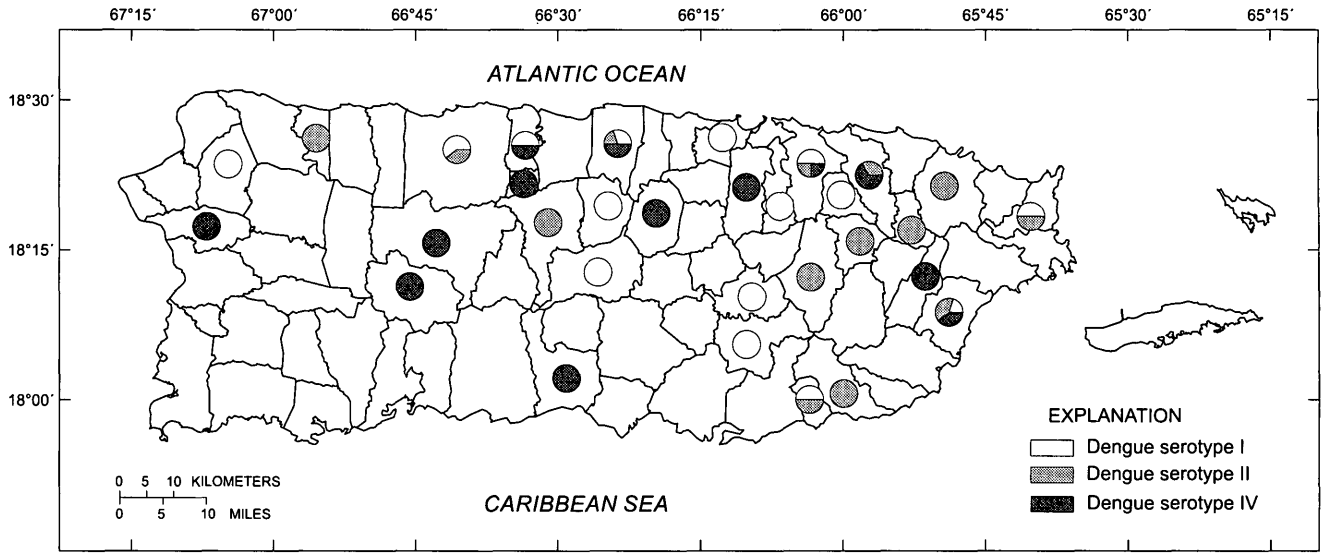
Appendix D5. Distribution of individual dengue serotype in Puerto Rico, October 1991.



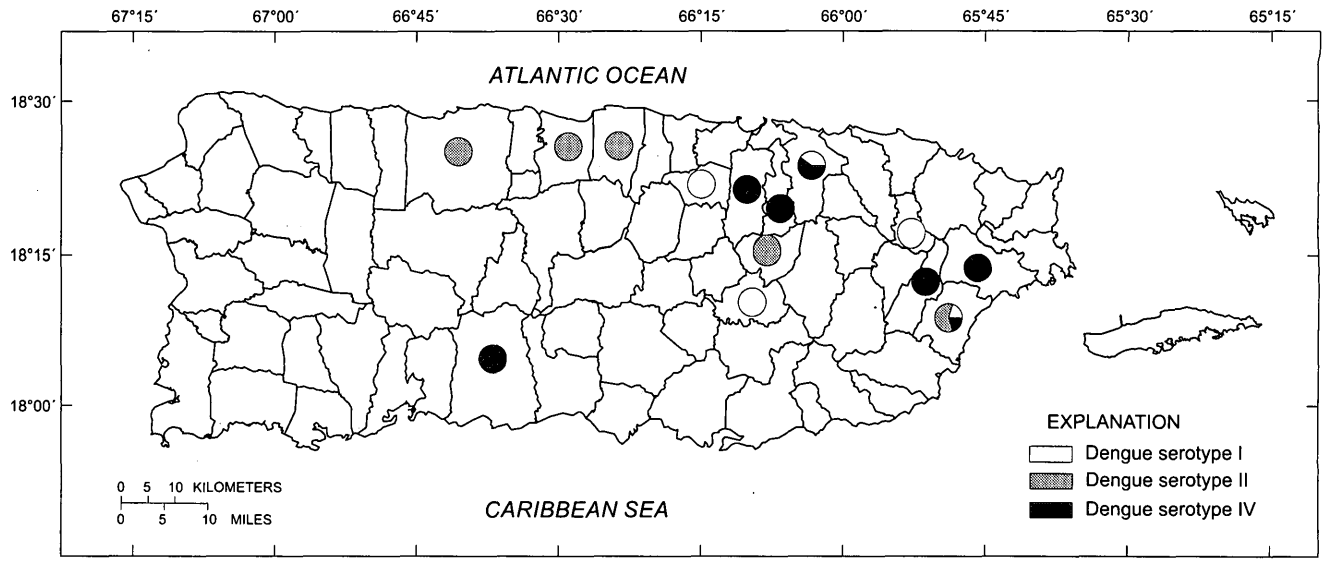
Appendix D6. Distribution of individual dengue serotype in Puerto Rico, November 1991.



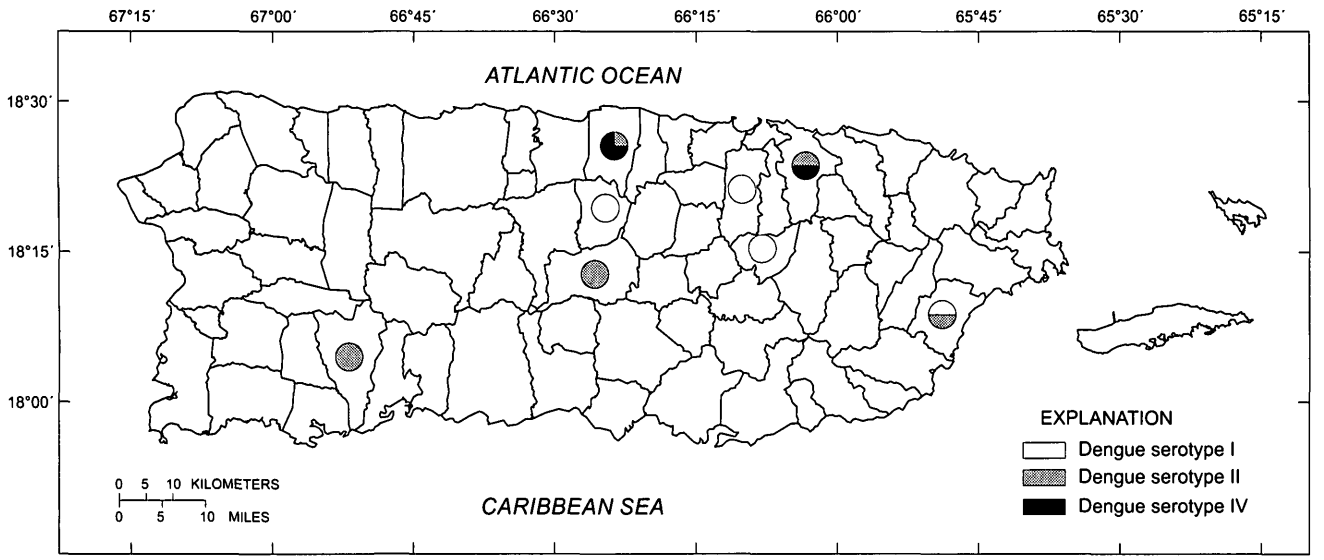
Appendix D7. Distribution of individual dengue serotype in Puerto Rico, December 1991.



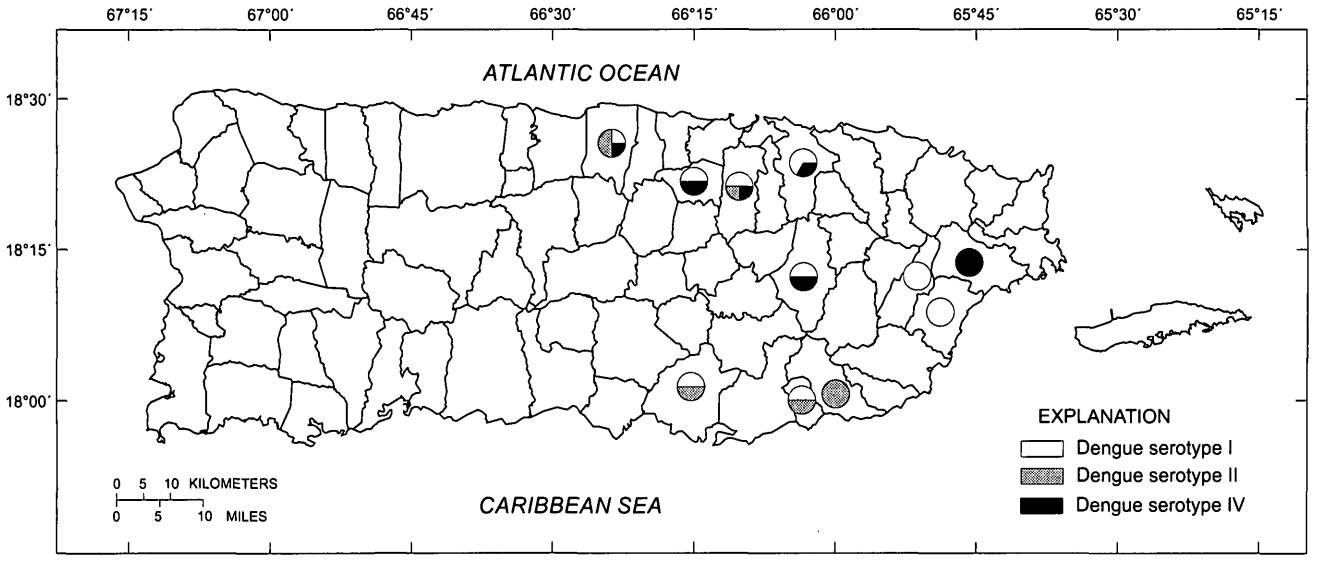
Appendix D8. Distribution of individual dengue serotype in Puerto Rico, January 1992.



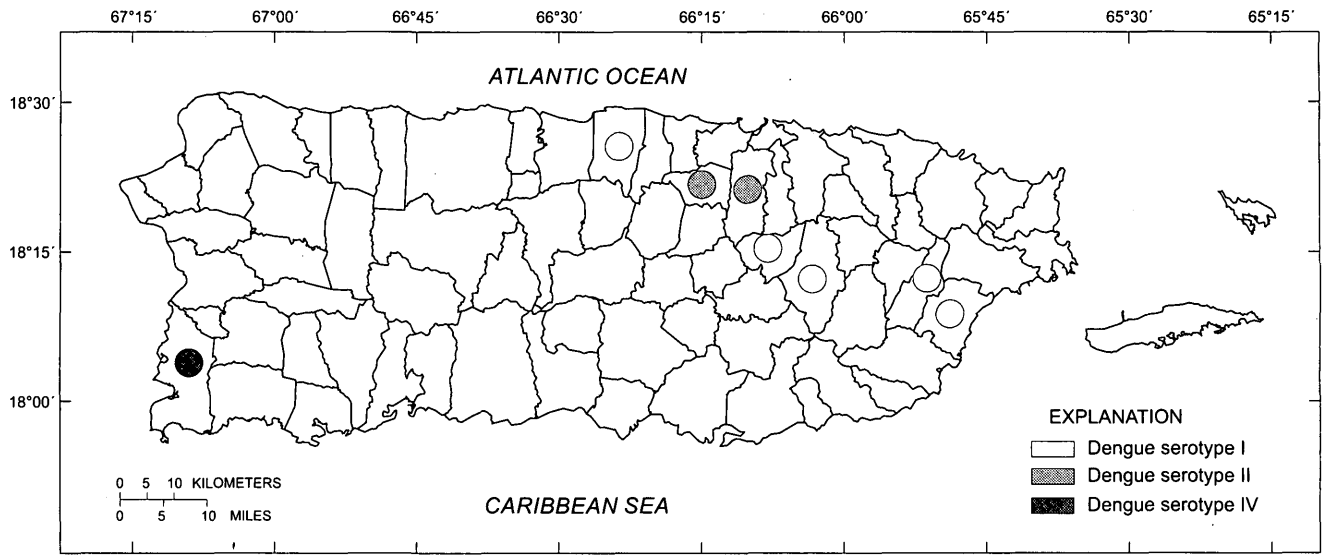
Appendix D9. Distribution of individual dengue serotype in Puerto Rico, February 1992.



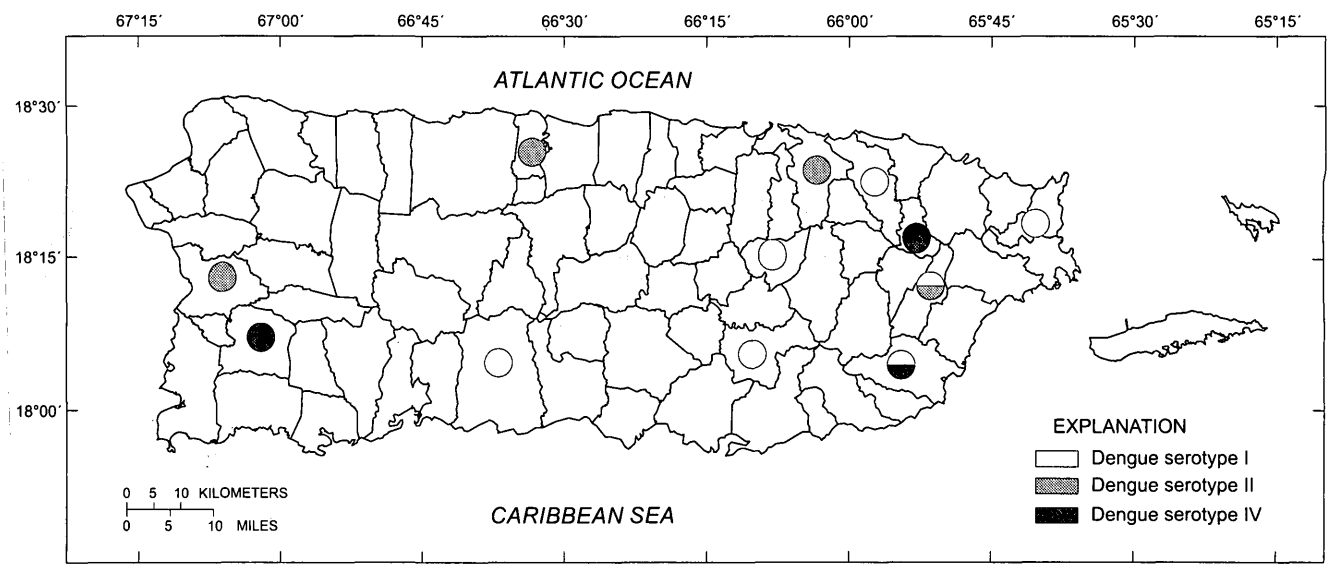
Appendix D10. Distribution of individual dengue serotype in Puerto Rico, March 1992.



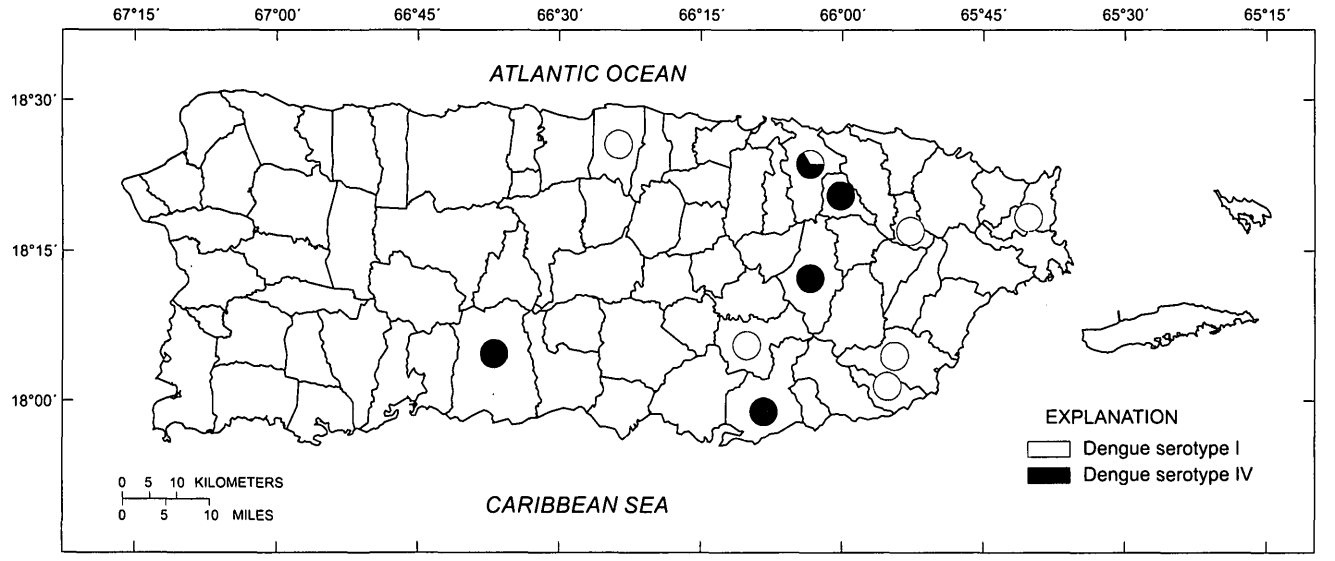
Appendix D11. Distribution of individual dengue serotype in Puerto Rico, April 1992.



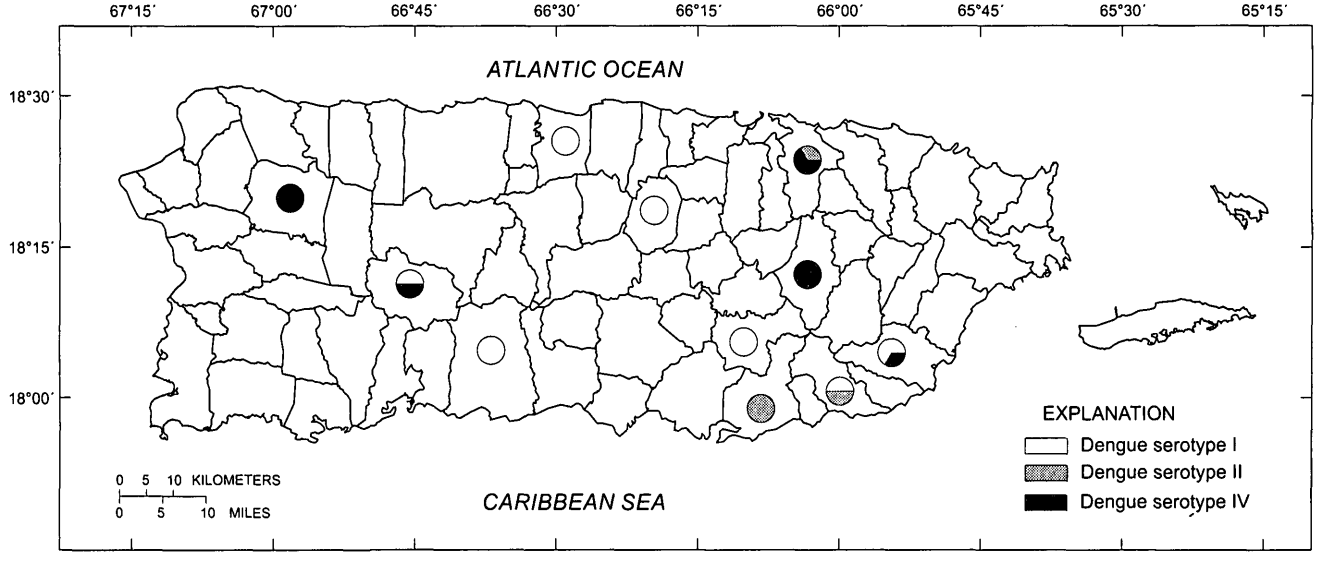
Appendix D12. Distribution of individual dengue serotype in Puerto Rico, May 1992.



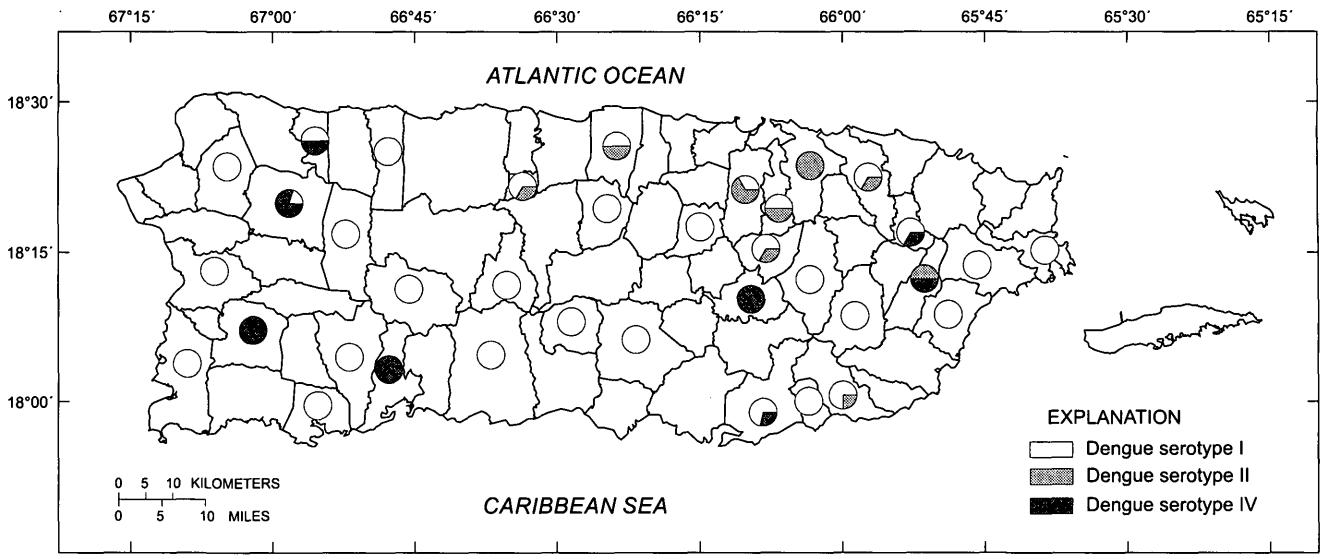
Appendix E1. Distribution of individual dengue serotype in Puerto Rico, June 1992.



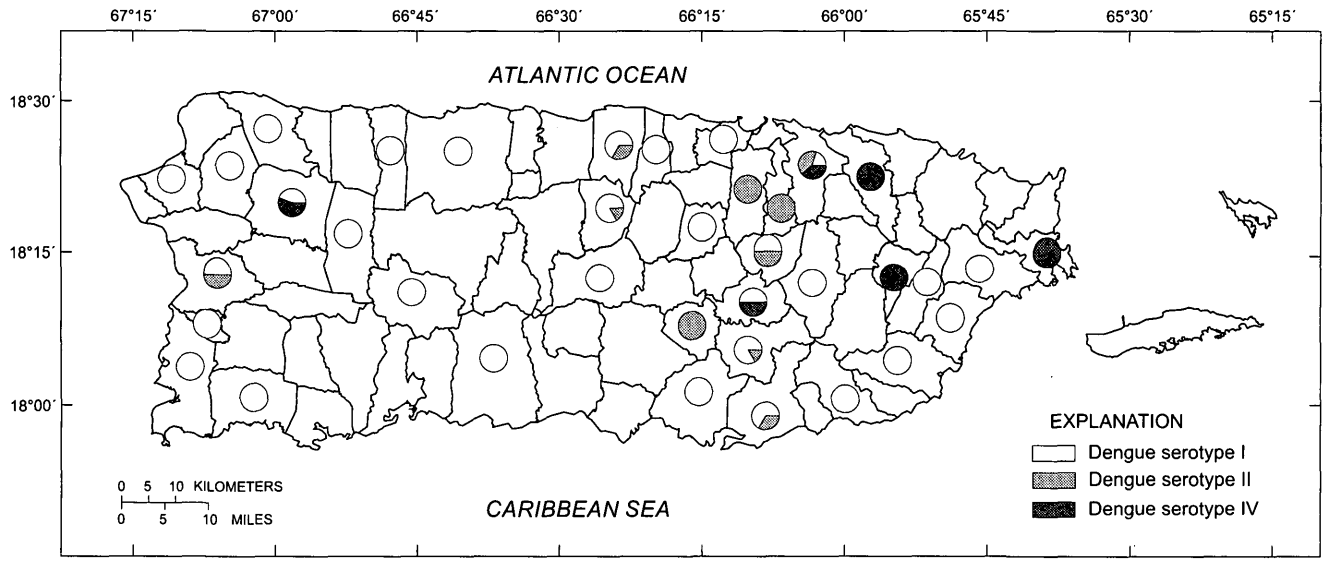
Appendix E2. Distribution of individual dengue serotype in Puerto Rico, July 1992.



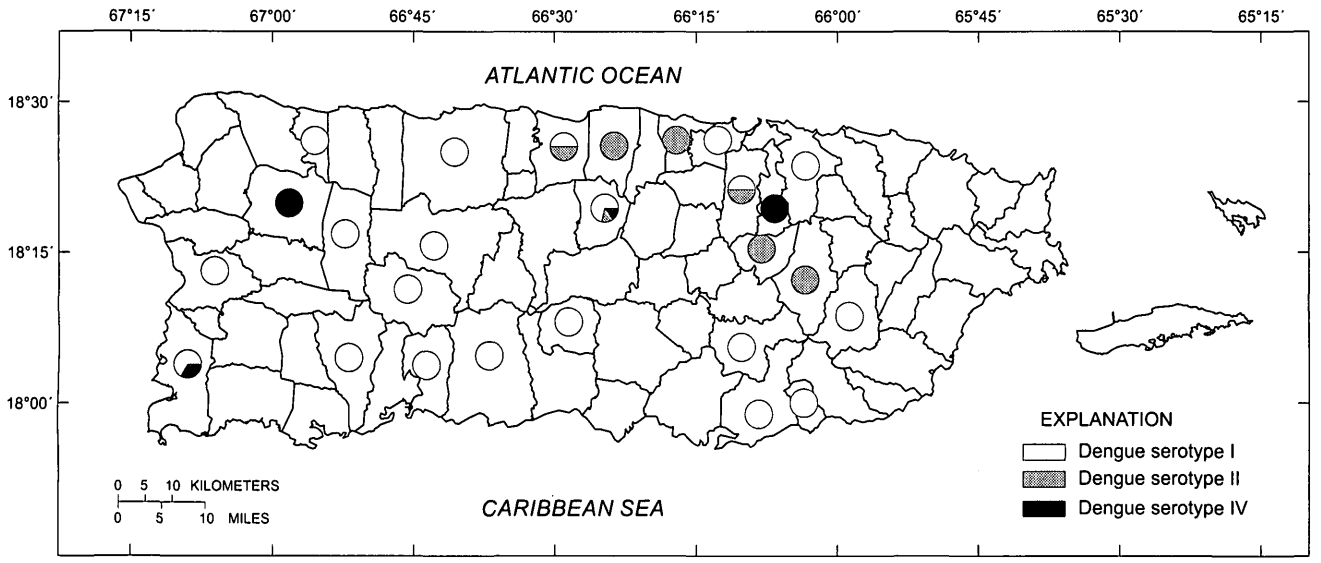
Appendix E3. Distribution of individual dengue serotype in Puerto Rico, August 1992.



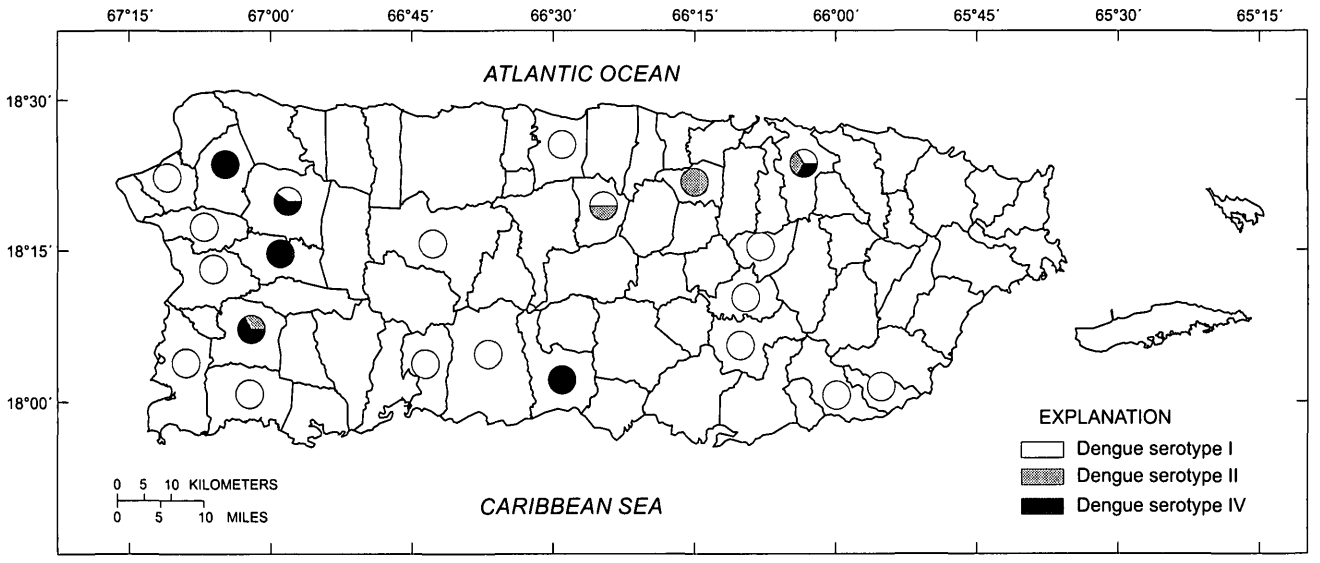
Appendix E4. Distribution of individual dengue serotype in Puerto Rico, September 1992.



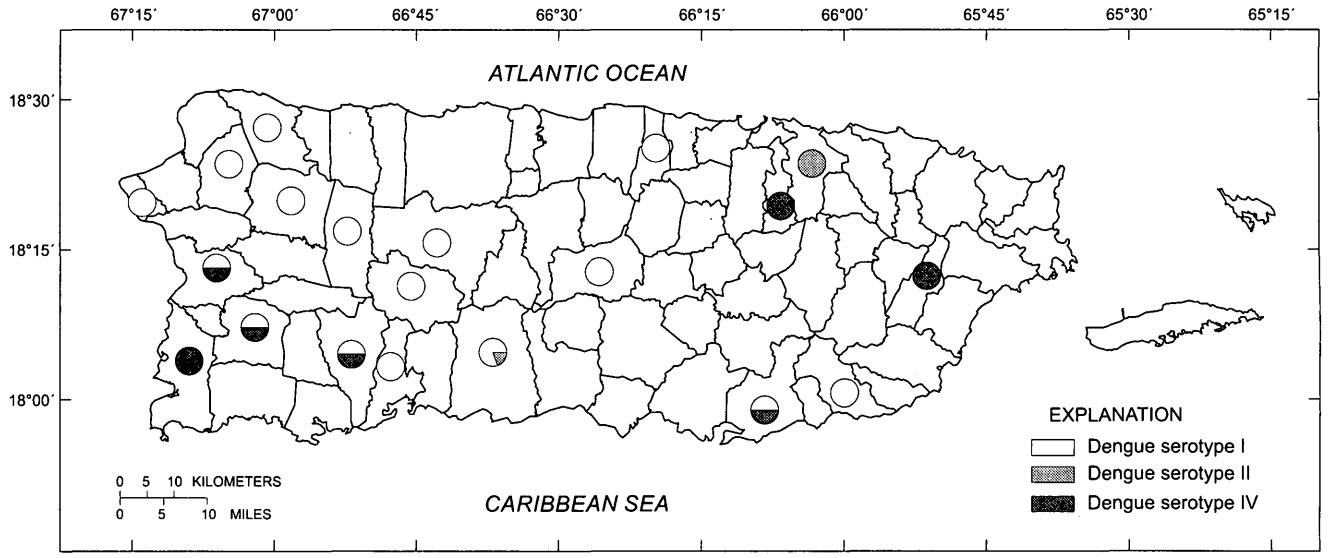
Appendix E5. Distribution of individual dengue serotype in Puerto Rico, October 1992.



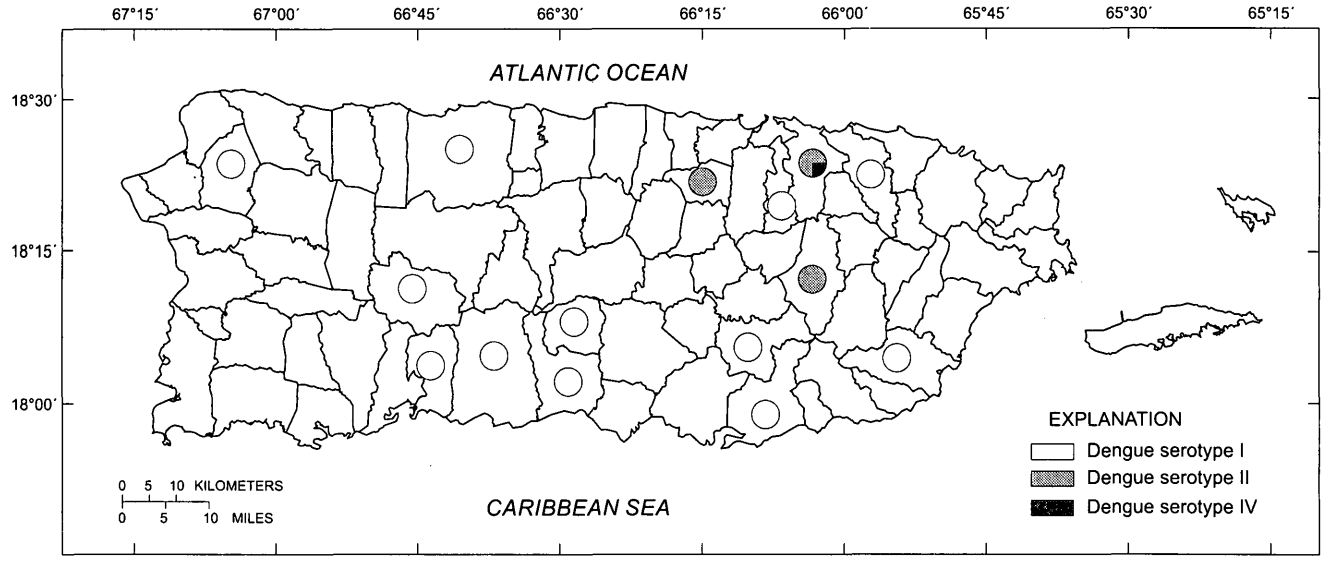
Appendix E6. Distribution of individual dengue serotype in Puerto Rico, November 1992.



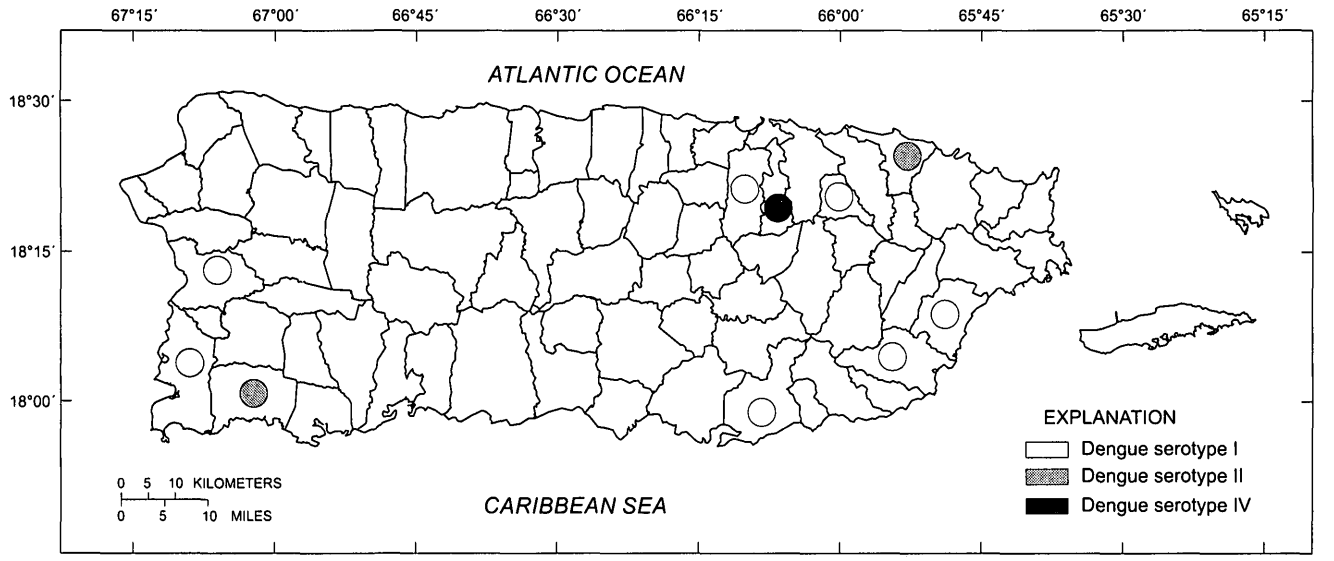
Appendix E7. Distribution of individual dengue serotype in Puerto Rico, December 1992.



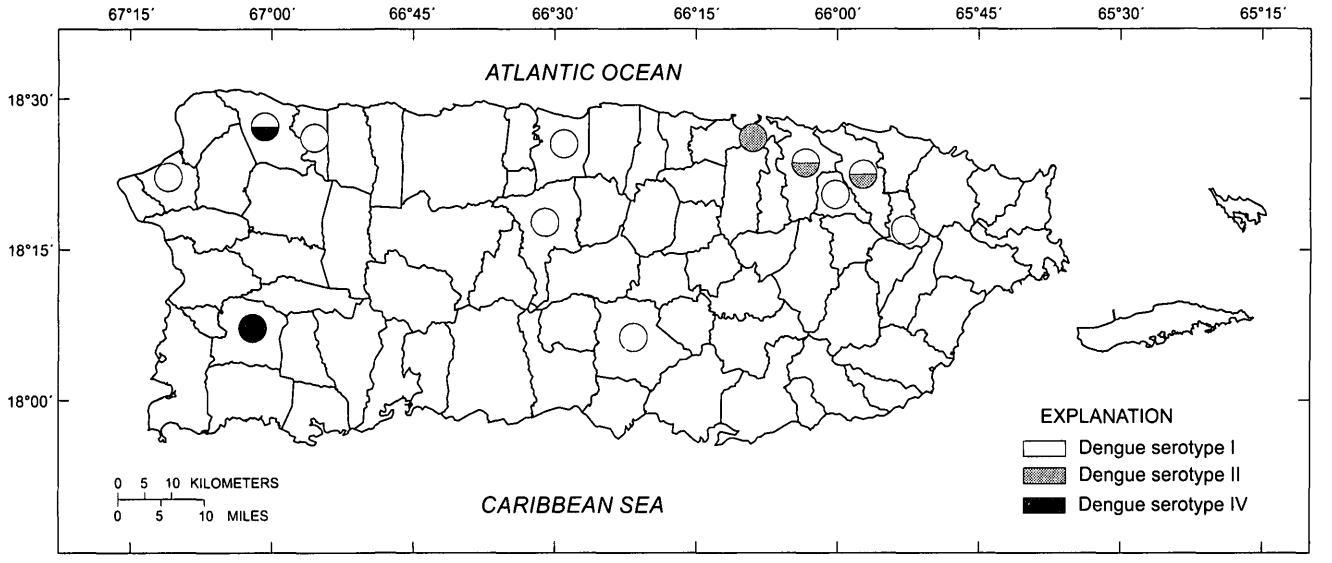
Appendix E8. Distribution of individual dengue serotype in Puerto Rico, January 1993.



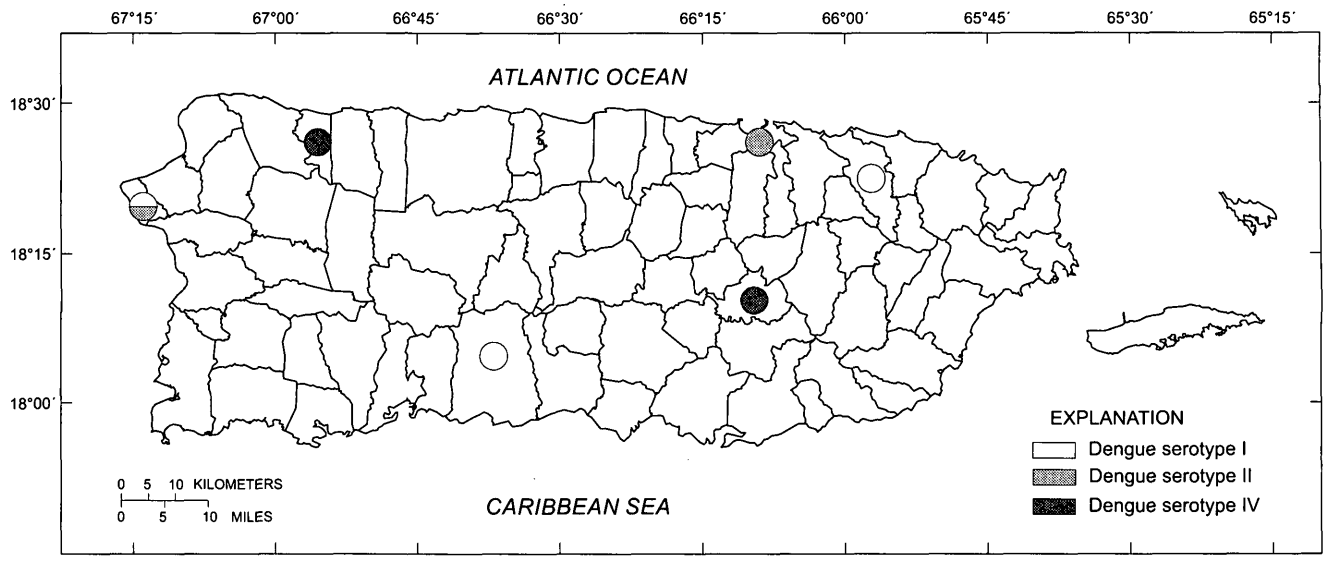
Appendix E9. Distribution of individual dengue serotype in Puerto Rico, February 1993.



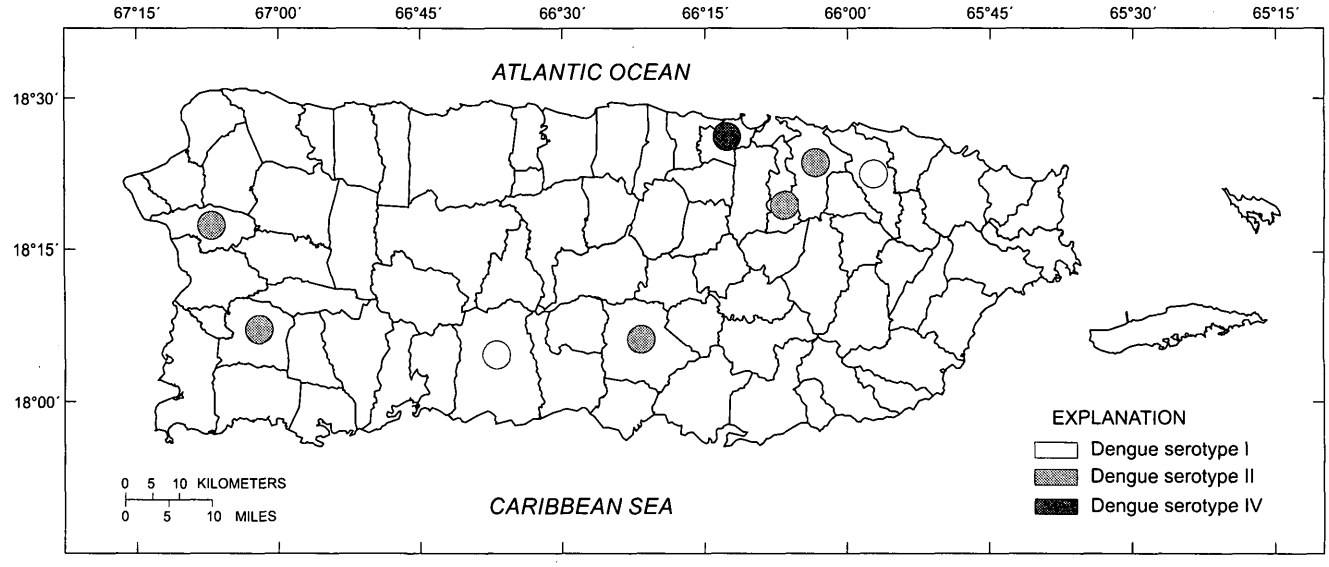
Appendix E10. Distribution of individual dengue serotype in Puerto Rico, March 1993.



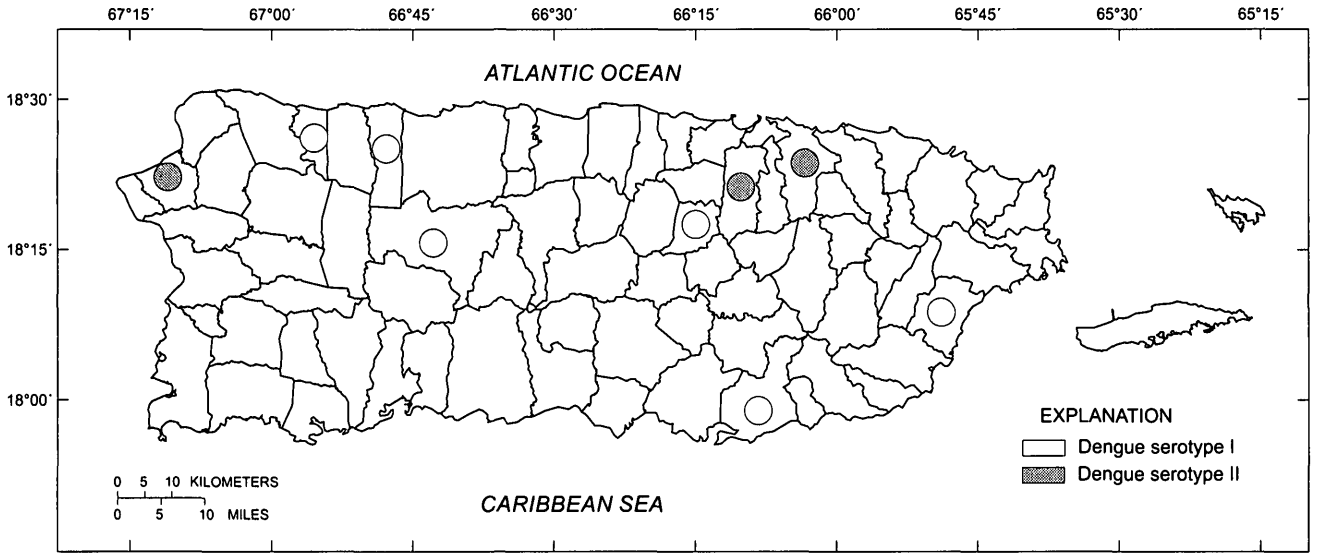
Appendix E11. Distribution of individual dengue serotype in Puerto Rico, April 1993.



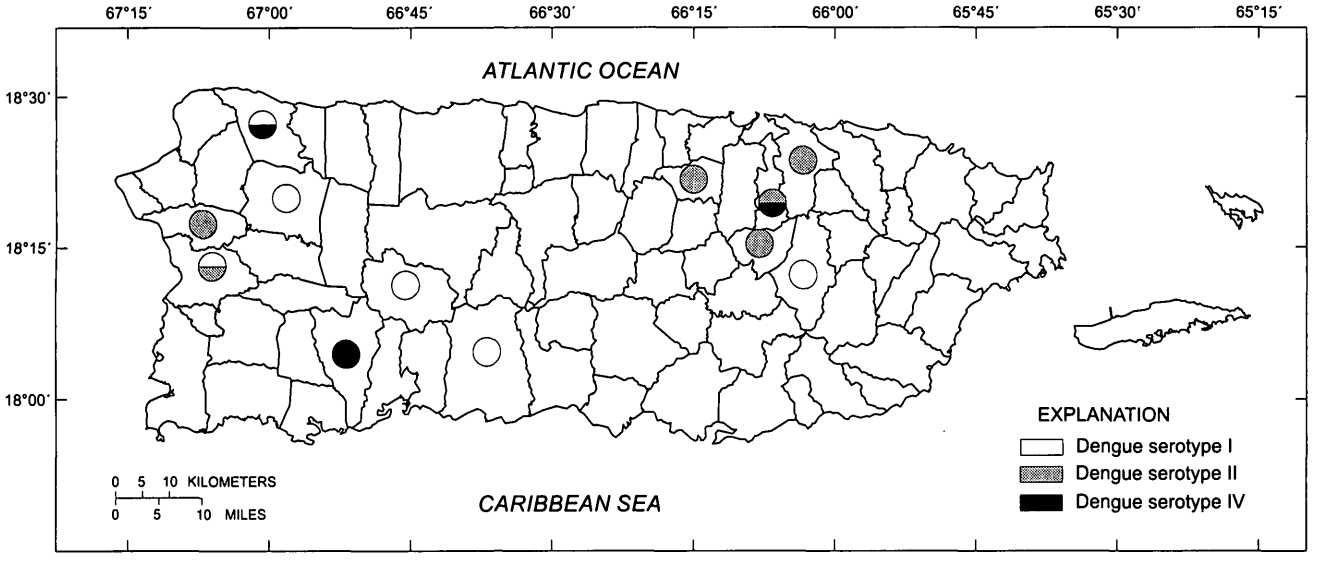
Appendix E12. Distribution of individual dengue serotype in Puerto Rico, May 1993.



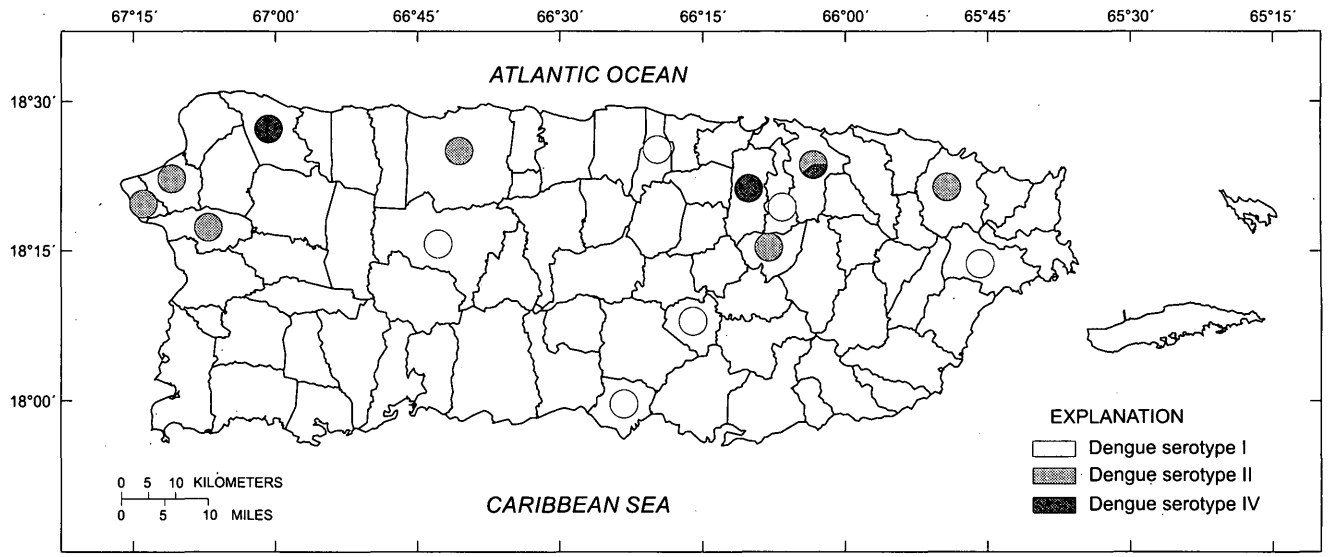
Appendix F1. Distribution of individual dengue serotype in Puerto Rico, June 1993.



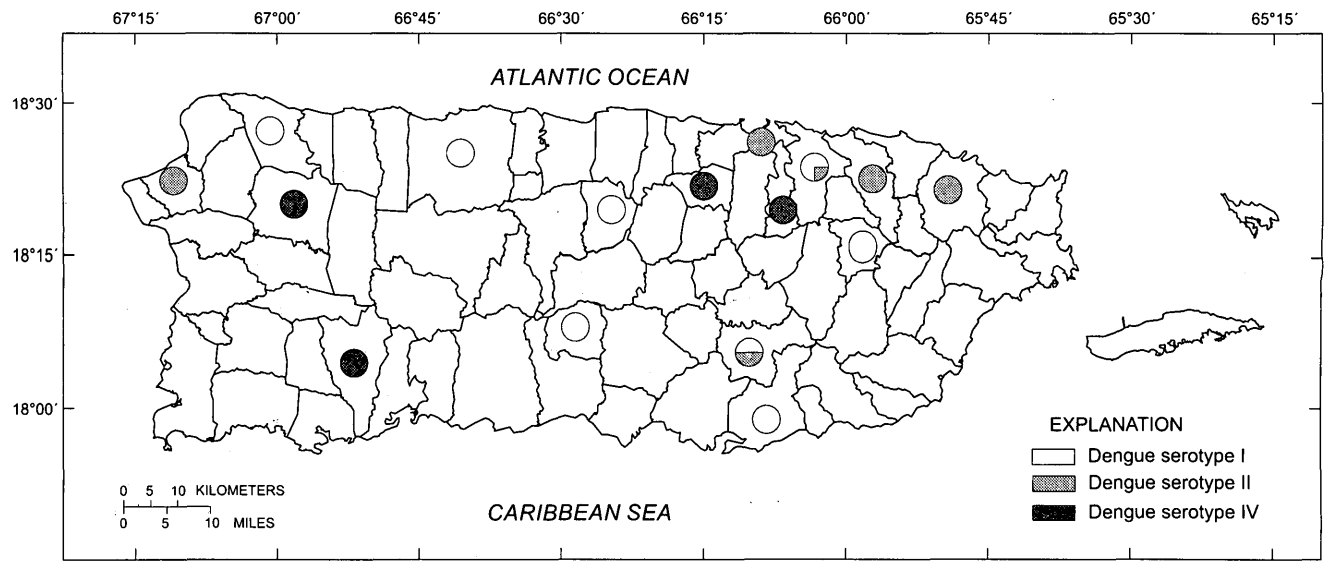
Appendix F2. Distribution of individual dengue serotype in Puerto Rico, July 1993.



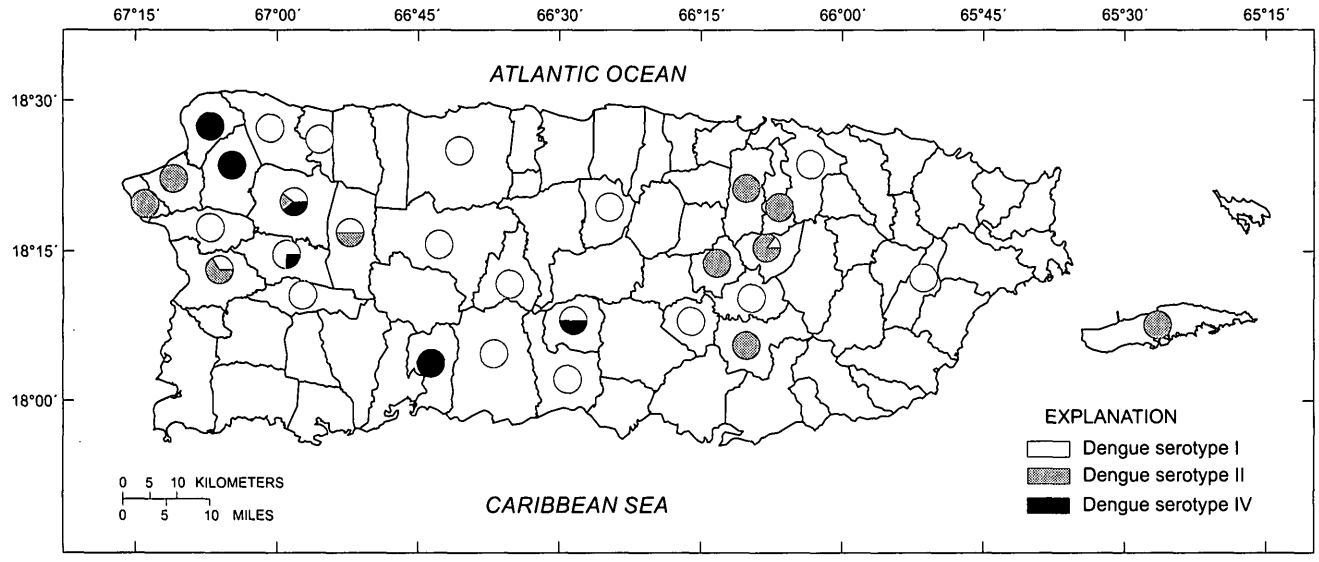
Appendix F3. Distribution of individual dengue serotype in Puerto Rico, August 1993.



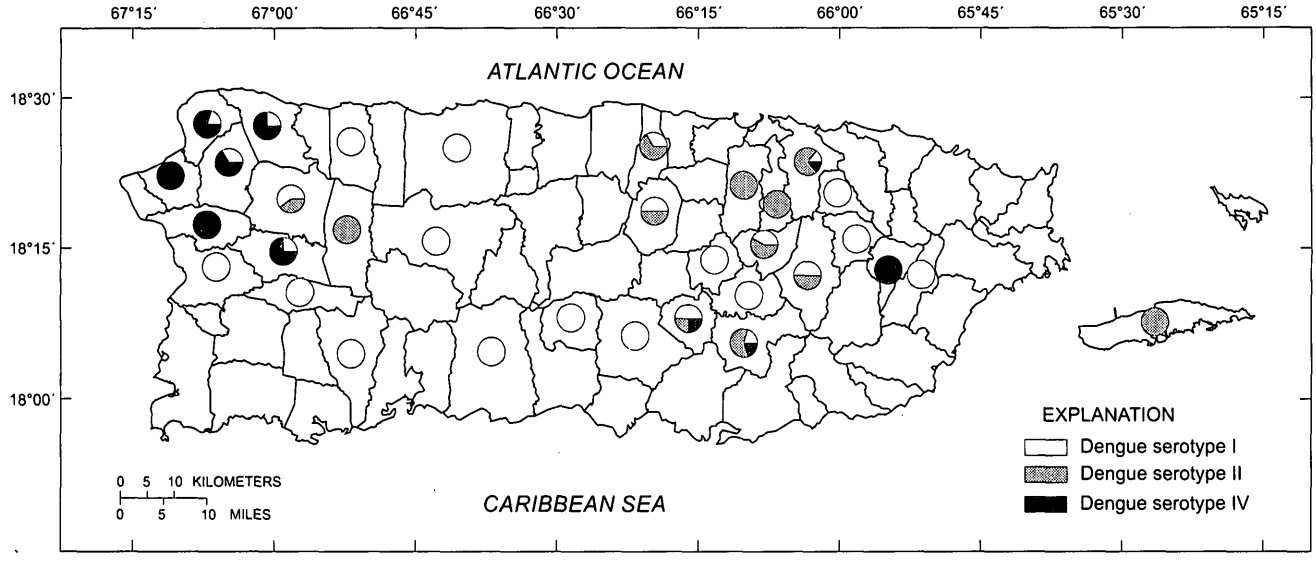
Appendix F4. Distribution of individual dengue serotype in Puerto Rico, September 1993.



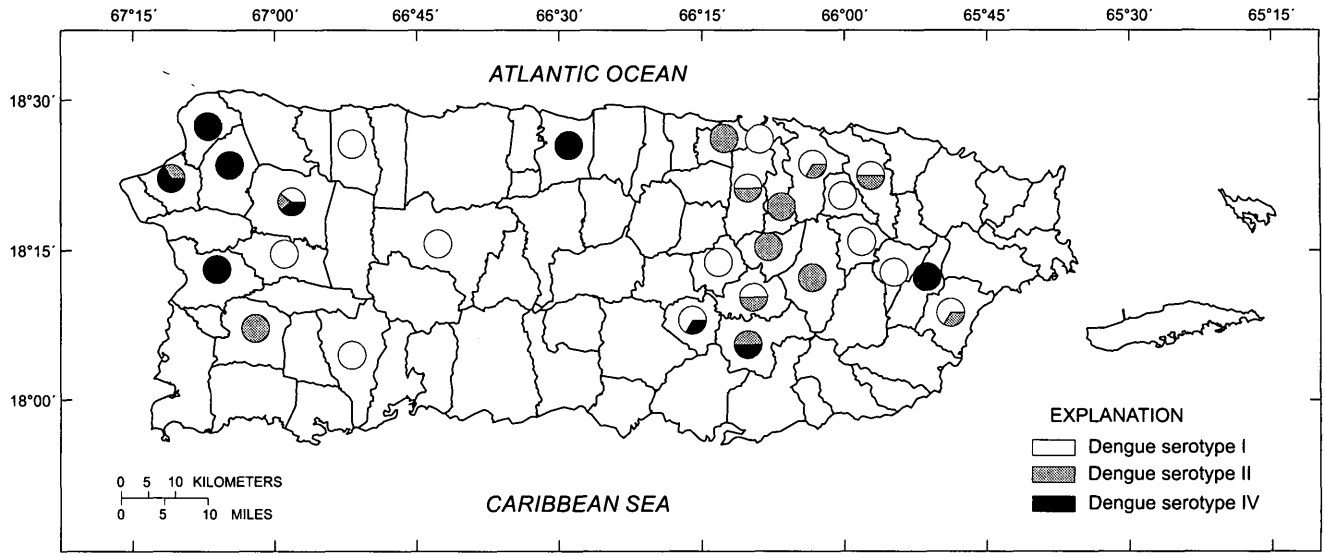
Appendix F5. Distribution of individual dengue serotype in Puerto Rico, October 1993.



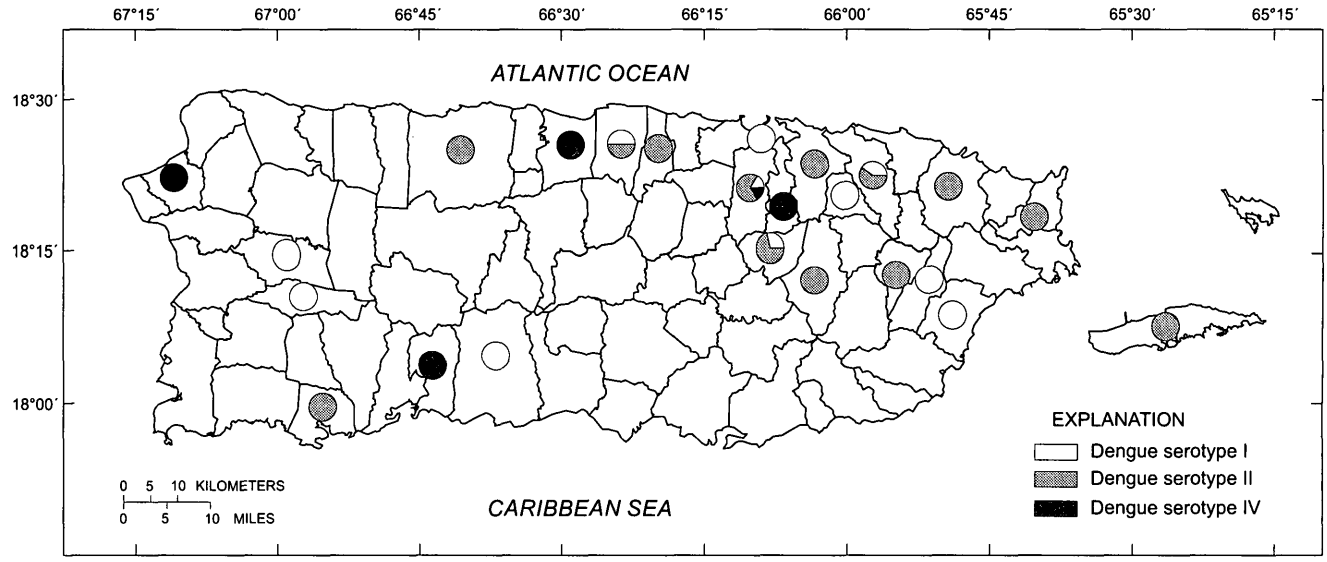
Appendix F6. Distribution of individual dengue serotype in Puerto Rico, November 1993.



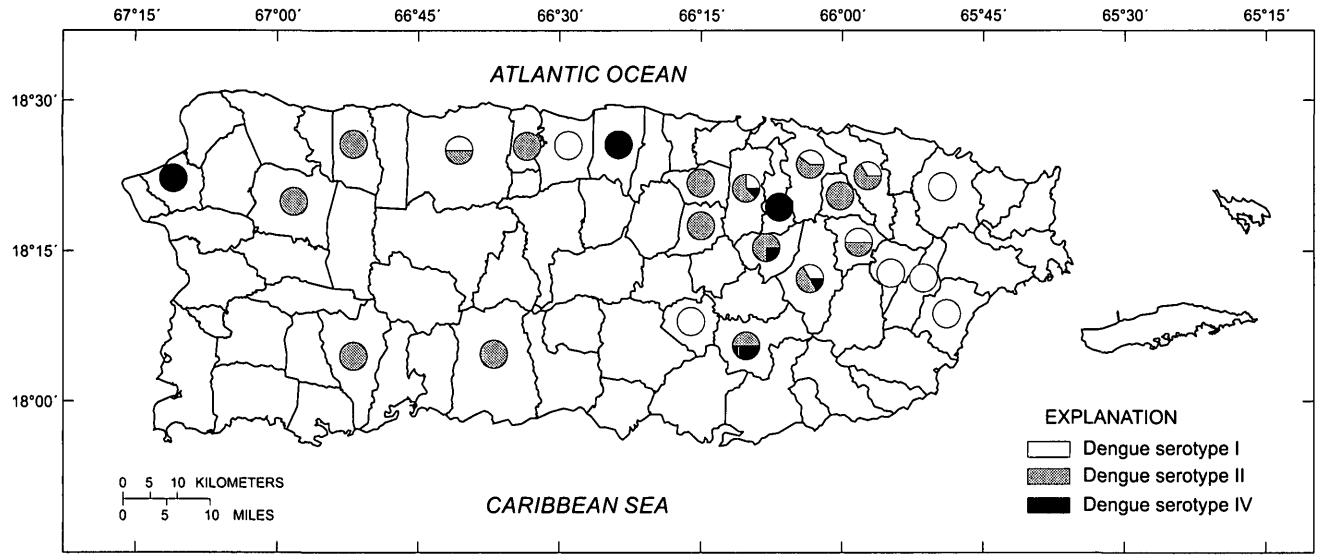
Appendix F7. Distribution of individual dengue serotype in Puerto Rico, December 1993.



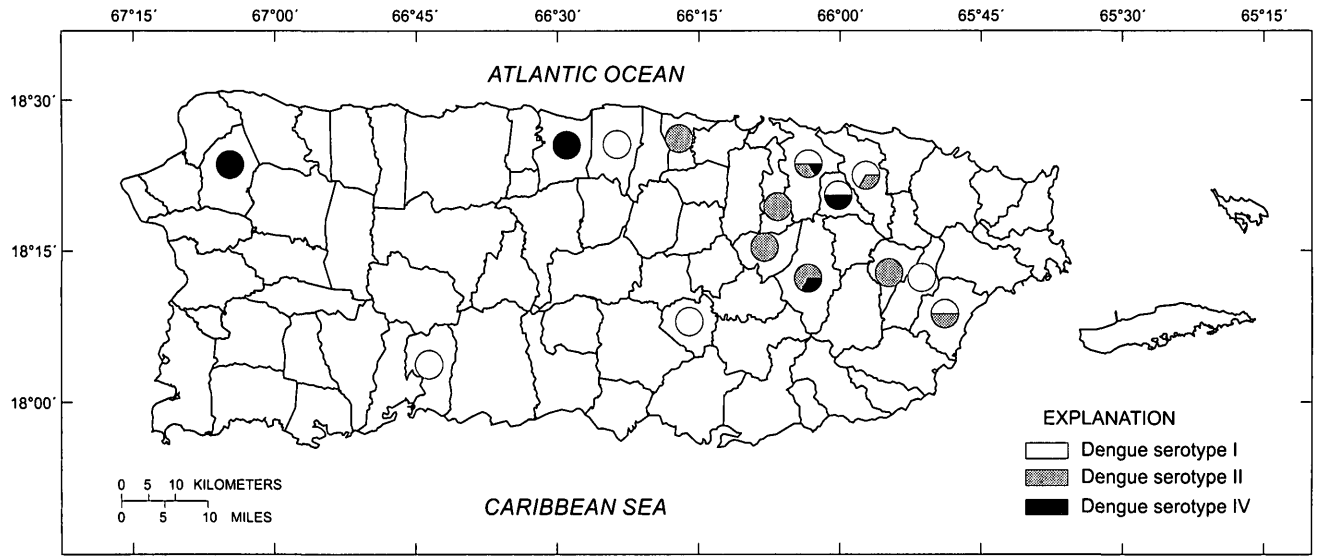
Appendix F8. Distribution of individual dengue serotype in Puerto Rico, January 1994.



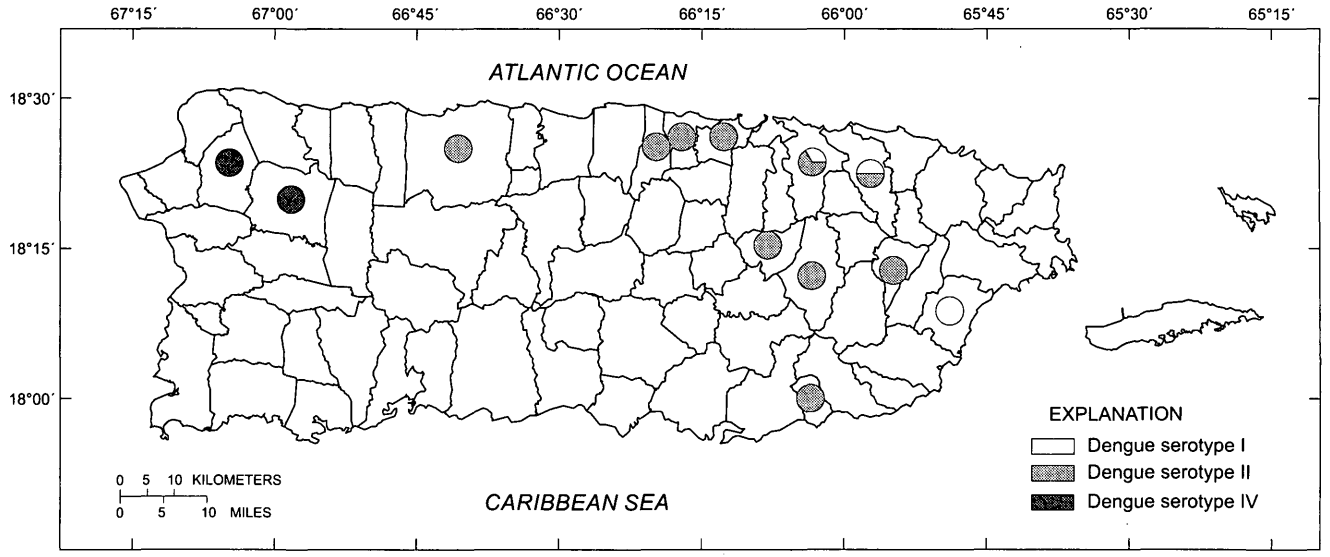
Appendix F9. Distribution of individual dengue serotype in Puerto Rico, February 1994.



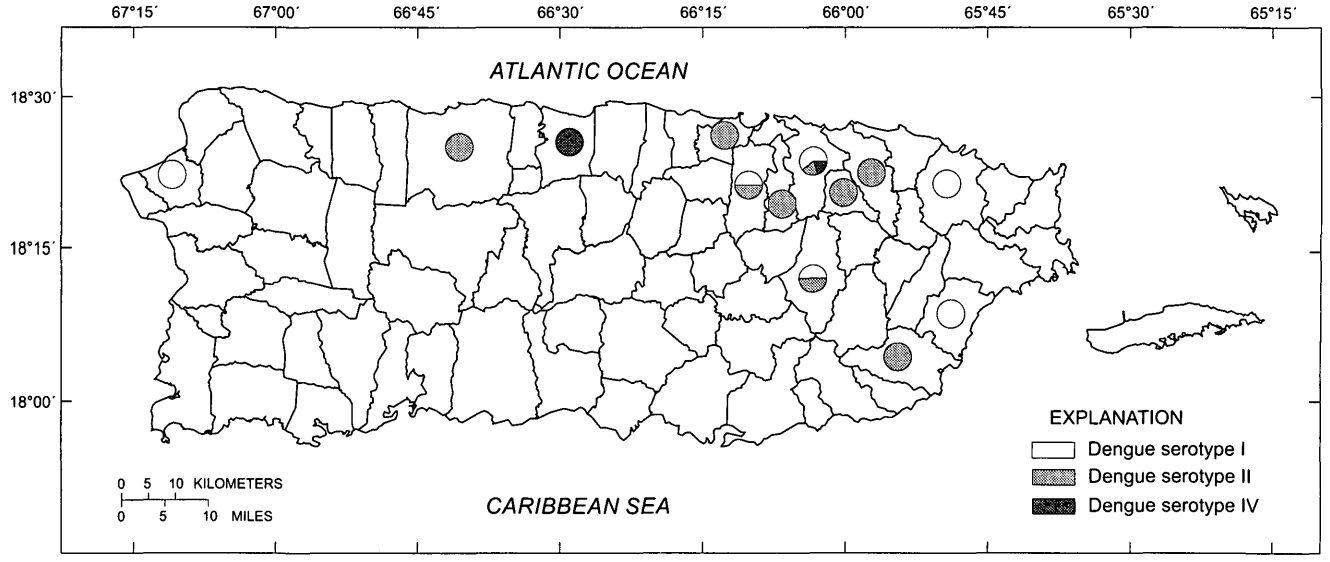
Appendix F10. Distribution of individual dengue serotype in Puerto Rico, March 1994.



Appendix F11. Distribution of individual dengue serotype in Puerto Rico, April 1994.



Appendix F12. Distribution of individual dengue serotype in Puerto Rico, May 1994.



District Chief
Caribbean District
U.S. Geological Survey
Water Resources Division
GSA Center, Suite 400-15
651 Federal Drive
Guaynabo, Puerto Rico 00965-5703