Revealing the microscale spatial signature of dengue transmission and immunity in an urban population

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It is well-known that the distribution of immunity in a population dictates the future incidence of infectious disease, but this process is generally understood at individual or macroscales. For example, herd immunity to multiple pathogens has been observed at national and city levels. However, the effects of population immunity have not previously been shown at scales smaller than the city (e.g., neighborhoods). In particular, no study has shown long-term effects of population immunity at scales consistent with the spatial scale of person-to-person transmission. Here, we use the location of dengue patients' homes in Bangkok with the serotype of the infecting pathogen to investigate the spatiotemporal distribution of disease risk at small spatial scales over a 5-y period. We find evidence for localized transmission at distances of under 1 km. We also observe patterns of spatiotemporal dependence consistent with the expected impacts of homotypic immunity, heterotypic immunity, and immune enhancement of disease at these distances. Our observations indicate that immunological memory of dengue serotypes occurs at the neighborhood level in this large urban setting. These methods have broad applications to studying the spatiotemporal structure of disease risk where pathogen serotype or genetic information is known.

dynamics | spatial statistics | dengue hemorrhagic fever

Individual risk of infectious diseases is largely determined by immune status and the rate of contact with infectious agents. Past infection or vaccination in an area can reduce infection risk for susceptible individuals by eliminating potentially infectious neighbors (1–6). Daily movements of host populations determine the spatial scale at which the immunity of neighbors is relevant for disease risk. Models that assume large-scale mixing will have homogenous levels of population immunity (7); however, there may be important differences at smaller scales (hundreds of meters) that affect the distribution of disease. Analysis of spatiotemporal locations of cases at fine resolutions may reveal the microscale dynamics of transmission and population immunity.

Dengue is a viral disease transmitted by the *Aedes* mosquito, with clinical manifestations ranging from asymptomatic illness to potentially fatal dengue hemorrhagic fever (8). Dengue is present in over 100 countries, causing an estimated 50 million infections and 19,000 deaths each year (9). A wide range of vector, human, viral, and environmental factors determine the spatial and temporal patterns of dengue infection (8). These factors include the spatial distribution and movement of mosquitoes and humans; life span, oviposition, and blood feeding tendencies of the mosquito; the infectiveness of both hosts; and the spatial distribution of immunity in humans (8). There are four serotypes of dengue virus (DENV1–4). All four have circulated in Bangkok, Thailand for decades (10). After infection, individuals develop lifelong immunity to the infecting serotype (homotypic immunity), and there is evidence that they are temporarily protected from infection with other serotypes (heterotypic immunity) (11). However, after susceptibility to other serotypes returns, these individuals are at increased risk of severe disease on infection (heterotypic immune enhancement) (12, 13).

Several studies have described the spatial clustering of dengue cases, but they did not explore the effect of population immunity (14–16). To our knowledge, the effect of population immunity on microscale disease dynamics has never been systematically characterized using empirical data. This lack of characterization is understandable, because direct observation of the spatial and temporal dynamics of cases and immunity is difficult and resource-intensive, requiring longitudinal observation of immune status and case incidence over large spatial and temporal scales. Here, we characterize the dynamics of population immunity and its effect on future incidence using only the spatiotemporal distribution of clinical dengue cases presenting at a single large hospital.

We use the household location of 1,912 children with laboratory-confirmed dengue illness admitted to Queen Sirikit Hospital, Bangkok between 1995 and 2000 to calculate measures of spatiotemporal dependence (Fig. 1). We use modifications of standard space–time clustering statistics that allow for finer resolution of spatiotemporal dependence and control for changes in the underlying spatial and temporal distribution of the population. This approach is built on an innovative use of the distribution of heterotypic case pairs (those cases inconsistent with transmission) and homotypic pairs (those cases consistent with transmission) over a long timescale to characterize the underlying spatial and temporal heterogeneity in disease risk. We use these methods to investigate whether the spatiotemporal distribution of cases is consistent with localized transmission, the expected effect of long-term homotypic immunity, short-term heterotypic immunity, and immune enhancement of disease severity in secondary heterotypic infections.

Results

Short-Term Clustering. Spatiotemporal dependence exists when the time and location of a case is affected by where and when other cases occur (17). We characterize the spatial dependence of homotypic cases within a 1-month time horizon as $\tau(d_1, d_2)$: the relative probability of a case occurring during the same month and within distance range d_1 to d_2 of a given case being homotypic compared with the probability of any other case in that month being homotypic (*Methods*). Both the numerator and denominator are dependent on the spatiotemporal distribution of cases appearing

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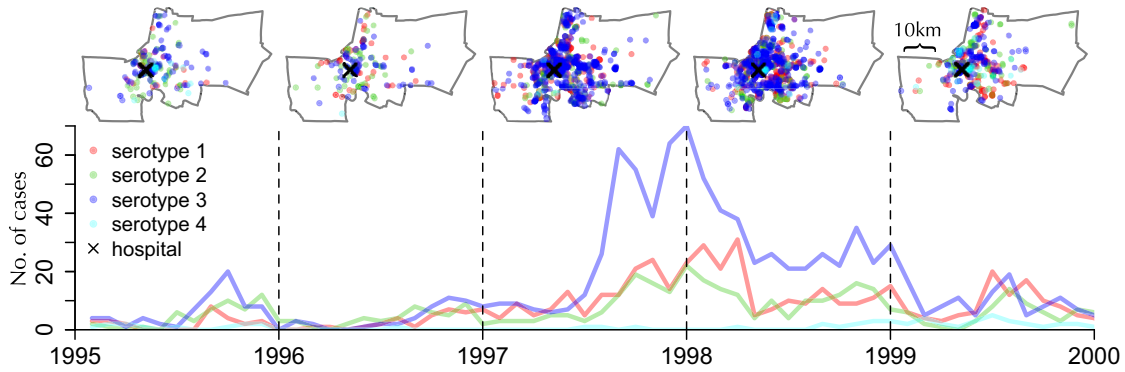

Fig. 1. Spatial and temporal distribution of clinical cases of dengue disease by month at Queen Sirikit Hospital between 1995 and 2000. The border in each map represents the Bangkok provincial boundary.

within the same month regardless of serotype. This formulation, therefore, controls for underlying heterogeneities in the population that could create spatial or temporal clustering (e.g., variation in population density, hospital and healthcare use rates, and dengue seasonality). Values above one indicate that any two cases that live within the specified distance range of each other are more likely to be homotypic than any two randomly chosen cases presenting during the same month. Cases coming from the same transmission chain are necessarily homotypic, and hence, spatial clustering of homotypic cases over short time periods may indicate transmission-related cases.

We find a 1.82-fold increase in the probability of a case occurring within 200 m and in same month of another case being homotypic (95% confidence interval of 1.45, 2.16) (Fig. 2). This estimate falls to 1.16 (0.97, 1.35) at 1 km (±250 m; i.e., the spatial range between 750 m and 1.25 km). There is an increased probability of cases being homotypic at distances up to 1.8 km (±250 m). However, this finding is statistically significant only up to 0.7 km (±250 m). Consistent patterns are observed with each of the four serotypes (Fig. S1 *B–E*). Consistent patterns are also found when only considering cases north, south, east, or west of the hospital (Fig. S2). These results suggest that the transmission of dengue in urban Bangkok is focal. Clustering of homotypic cases may be caused by local dispersal of host and vector. However, clustering of immune status in the population may contribute to focal case distributions.

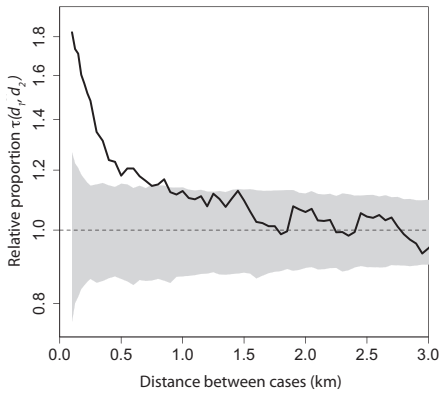

Fig. 2. Homotypic spatial dependence analysis for cases occurring within the same month. The size of the spatial window of analysis ($d_2 - d_1$) is kept at 0.5 km when d_2 is greater than 0.5 km. When d_2 is less than 0.5 km, d_1 is equal to zero. Estimates are plotted at the midpoint of the spatial range. The shaded area represents 95% confidence intervals of a null distribution generated from 1,000 simulations, where the time point at which a case occurs is randomly reassigned.

Longer-Term Clustering. The immune profile of the population can induce both short- and long-term spatiotemporal dependence in dengue cases. If we assume that neighborhood composition remains mostly the same within the study period and that detected cases are representative of serotype-specific incidence in that neighborhood, we would expect clustering of a particular serotype to result in a reduction in future homotypic cases in that vicinity. Likewise, during the period of short-term cross-protection, we expect to see fewer heterotypic cases occur near previous dengue cases. Conversely, immune enhancement may lead to increases in heterotypic cases at longer temporal lags (13).

Spatiotemporally dependent processes are often described using $D_0(d, t)$, which estimates the probability of a point occurring within a spatiotemporal distance of another point compared with the probability of this occurrence because of the independent effects of clustering in space and time (18–21). $D_0(d, t)$ is a cumu lative function; hence, it can only crudely characterize changing patterns of spatiotemporal dependence (Fig. S3). Thus, we derive a related function, $\Phi(d_1, d_2, t_1, t_2)$, of the relative probability of a homotypic (or heterotypic) case being within a window of space and time from a case versus the expectations if the clustering processes in space and time were independent (*Methods*).

Patterns of both homotypic and heterotypic spatiotemporal dependence differ substantially from those patterns seen if we ignore serotype (Fig. 3). We find that homotypic cases are 1.61 (1.42, 1.82) times as likely to occur within 400 m and 4 mo of an incident case than would be expected if the spatial and temporal clustering processes were independent (Fig. 3*B*). The relative proportion of homotypic cases falls to 1.14 (1.05, 1.23) at 1 km (±500 m) over the same timeframe. This period is followed by a significant reduction in homotypic cases in subsequent months. Homotypic cases are 0.77 (0.67, 0.86) times as likely to occur at temporal lags of 8–24 mo within 400 m and 0.90 (0.84, 0.96) times as likely at 1 km (±500 m) over the same temporal lags.

We find that heterotypic cases are 0.88 (0.85, 0.96) times as likely to occur at 1 km (±500m) from an incident case at lags of 3–10 mo (Fig. 3*C*). These heterotypic patterns are consistent with the findings in the work by Sabin (11) of short-lived cross-protective immunity. Furthermore, there is an increase in heterotypic clustering with a temporal lag of 2 y. Heterotypic cases are 1.11 (1.02, 1.20) times as likely to occur at temporal lags of 20–30 mo when 1 km (±500 m) from an incident case. This increase after a period of 2 y points to elevated risk of disease and supports previous observations of increased disease risk with sequential heterotypic infections (12).

Our analysis includes hospitalized cases only. The spatiotemporal dependence of hospitalized cases is of intrinsic interest. However, the mechanisms that we propose to explain the pattern of dengue cases rely on a correlation between the spatiotemporal

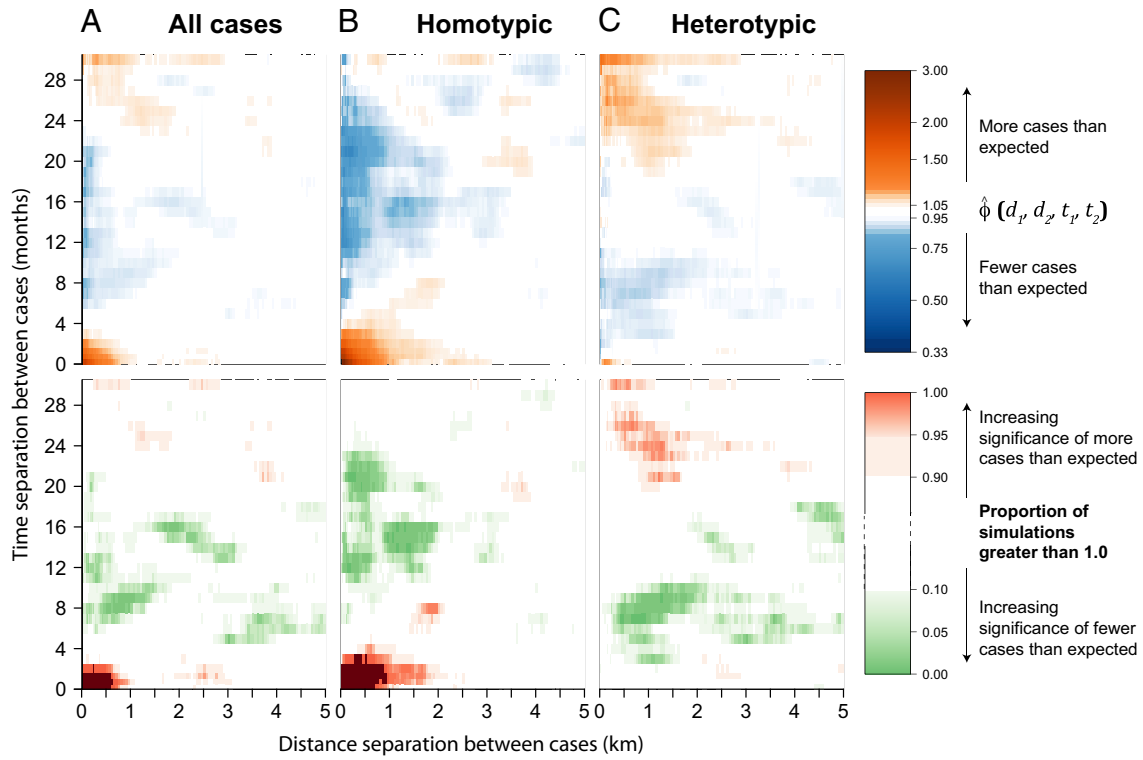

Fig. 3. Spatial dependence analysis with temporal lags. The average relative proportion of (*A*) all (irrespective of serotype), (*B*) homotypic, and (*C*) heterotypic cases is illustrated across spatial and temporal lags in the top row. The spatial range ($d_2 - d_1$) is kept constant at 0.5 km when d_2 is greater than 0.5 km. When d_2 is less than 0.5 km, d_1 is equal to zero. The temporal range ($t_2 - t_1$) is kept constant at 3 mo when t_2 is greater than 3 mo. When t_2 is less than 3 mo, t_1 is equal to zero. Estimates are plotted at the midpoint of the spatial and temporal ranges. $\hat{\Phi}(d_1, d_2, t_1, t_2)$ estimates under 5% in either direction are colored white. The bottom sets out the percentage of 1,000 bootstrapped simulations that have a $\hat{\Phi}(d_1, d_2, t_1, t_2)$ value greater than 1.0.

distribution of serotypes in hospitalized cases and the spatiotemporal distribution of serotypes in the unobserved cases from which these conclusions are drawn. We cannot establish this link with this dataset. However, we use two distinct statistics (analogous results to Figs. 2 and 3 are shown in Figs. S4 and S5). These statistics give unbiased estimates even in the presence of bias in reporting and heterogeneity in the underlying population density (Figs. S6 and S7). In addition, we simulated disease transmission processes where there were no spatial dependences between the infected and infecting individuals under conditions of strong seasonal forcing and no underlying heterogeneities in the distribution of the population. The ϕ- and τ-statistics showed no spatial dependence in these scenarios (Fig. S8). We describe the performance of our statistics in a number of simulated datasets in *SI Methods*. We find that our statistics are unbiased under a number of model scenarios. Movement of individuals into or out of the population by birth, death, immigration, or emigration may dilute the effect of acquired immunity on future cases. The period that a neighborhood remains effectively static will determine, in part, the extent to which we observe spatiotemporal dependence. Georeferencing of patient addresses in a large urban center is not without error. In sensitivity analyses, the signal of spatial clustering remains present in the actual data with the addition of random noise of a mean of 1 km. Errors in georeferencing are unlikely to be differential by serotype; hence, they would likely bias any results to the null and are unlikely to lead to spurious clustering.

Discussion

These results are from a large georeferenced dataset over several years in an endemic setting where all four serotypes circulate. Such datasets present a rare opportunity to study the dynamics of dengue transmission at a fine spatial resolution. Previous studies that have examined the spatial distribution of dengue have either focused on individual serotypes or have not had fine spatial resolution for large numbers of cases over multiple years (15, 16, 22, 23). Our study provides additional evidence to support the focal nature of DENV, complementing a longitudinal study in Thailand and outbreak investigations in other settings (14, 24, 25). Our results suggest that transmission is spatially local, even in a highly mobile and dense urban population with significant immunity. Our findings that the distribution of cases at one time point predict the spatial distribution of both homotypic and heterotypic cases at future time points suggest a dispersal mechanism that is partially dictated by the immunity status of the local population. Although the impact of population immunity on pathogen dynamics has been the focus of numerous studies, rarely has the spatiotemporal distribution of cases and importantly, the immunity derived from those cases been shown to predict the future distribution of cases.

Using multiple simulations, we show that it is unlikely that the observed clustering could be caused by underlying spatial structures, either in the population or access to the study hospital. In addition, we have shown that our observations could not be

Table 1. Characteristics of dengue cases

	N (*N* geocoded)	Percent
DENV1	571 (486)	25
DENV2	474 (406)	21
DENV3	1,142 (964)	51
DENV4	67 (56)	3
Total	2,254 (1,912)	
Secondary infections	1,740	77
DHF	1,654	73

Mean age was 7.5 y. DHF, dengue hemorrhagic fever.

generated solely by seasonal dynamics of dengue. Our results provide strong evidence that the clustering process is serotype-dependent. We believe that the most likely and simplest mechanism that would generate serotype-specific clustering is the transmission process, which we know to be serotype-dependent.

The methods implemented here use variations in pathogen type to characterize the tendency for cases to be found near each other both in the short term and across temporal lags. We use a passively collected dataset to illustrate how, by focusing on differences between event types, such datasets can be used to understand the underlying generating process. These approaches are relevant whenever there exists points of multiple types (e.g., genotype data) or changing patterns of spatiotemporal dependence not captured by a cumulative characterization, regardless of the domain. Here, these methods have revealed microscale interactions between transmission, immunity, and future incidence of dengue.

Methods

Data Collection. Data on clinical cases of dengue between January 1, 1995 and December 31, 1999 were collected from Queen Sirikit Children's Hospital in Bangkok, Thailand. There are a total of 2,254 cases where address, infecting serotype, and month and year of hospital admission are available (Table 1). Serotype was determined through RT-PCR. Local data managers used base maps for the city to convert addresses to geocoded point locations for each case.

Short-Term Spatial Dependence Analysis. To characterize the spatial dependence of homotypic cases within a 1-mo timeframe, we calculate the relative probability of a case occurring during the same month and within distance range d_1 to d_2 of a given case being homotypic compared with the probability of any other case in that month being homotypic (Eq. **1**):

$$\tau(d_1, d_2) = \frac{\Pr\left(z_i = z_j \,|\, j \in \Omega_i(d_1, d_2)\right)}{\Pr\left(z_i = z_j \,|\, j \in \Omega_i(\cdot)\right)}, \qquad [1]$$

where $\Omega_i(d_1, d_2)$ is the set of cases occurring during the same month and within distances d_1 and d_2 of case i; $\Omega_i(\cdot)$ is the set of all cases occurring in the same month, and z_i is the serotype of case i.

$\tau(d_1, d_2)$ is estimated as (Eq. **2**)

$$\hat{\tau}(d_1, d_2) = \frac{\sum_{i=1}^{N}\sum_{j\in\Omega_i(d_1,d_2)} z_{ij}}{\sum_{i=1}^{N}|\Omega_i(d_1, d_2)|} \Bigg/ \frac{\sum_{i=1}^{N}\sum_{j\in\Omega_i(\cdot)} z_{ij}}{\sum_{i=1}^{N}|\Omega_i(\cdot)|}, \qquad [2]$$

where z_{ij} is equal to one if the serotype of case i is equal to the serotype of case j and equal to zero otherwise. $\tau(d_1, d_2)$ is equivalent to a ratio of modified space–time K functions (17, 19, 21).

Long-Term Spatial Dependence Analysis. To calculate the spatial dependence over several months or years, we calculate the relative probability of a homotypic (or heterotypic) case being within a window of space and time from a case vs. the occurrence expected if the clustering processes in space and time were independent (Eq. **3**):

$$\Phi_{hom}(d_1, d_2, t_1, t_2) = \frac{\Pr\left(j \in \Omega_i(d_1, d_2, t_1, t_2) | z_i = z_j\right)}{\Pr\left(j \in \Omega_i(d_1, d_2, \cdot) | z_i = z_j\right)\Pr\left(j \in \Omega_i(\cdot, t_1, t_2) | z_i = z_j\right)}. \qquad [3]$$

$\Omega_i(d_1, d_2, t_1, t_2)$ is the set of cases within distances d_1 and d_2 and time range t_1 and t_2 of case i.

Φ_{hom} is estimated as (Eq. **4**)

$$\hat{\Phi}_{hom}(d_1, d_2, t_1, t_2) = \frac{\left(\sum_{i=1}^{N}\sum_{j\in\Omega_i(\cdot,\cdot,\cdot,\cdot)} z_{ij}\right)\cdot\left(\sum_{i=1}^{N}\sum_{j\in\Omega_i(d_1,d_2,t_1,t_2)} z_{ij}\right)}{\left(\sum_{i=1}^{N}\sum_{j\in\Omega_i(\cdot,t_1,t_2)} z_{ij}\right)\cdot\left(\sum_{i=1}^{N}\sum_{j\in\Omega_i(d_1,d_2,\cdot)} z_{ij}\right)}. \qquad [4]$$

The function for heterotypic cases is similarly estimated.

Additional descriptions of the spatiotemporal dependence methods used and their relationship with existing methodologies can be found in *SI Methods*.

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1. Anderson R, May RM (1991) *Infectious Diseases of Humans* (Oxford Science Publications, Oxford).
2. Grenfell BT, Bjørnstad ON, Kappey J (2001) Travelling waves and spatial hierarchies in measles epidemics. *Nature* 414:716–723.
3. Wallinga J, Teunis P, Kretzschmar M (2003) Reconstruction of measles dynamics in a vaccinated population. *Vaccine* 21:2643–2650.
4. Grassly NC, Fraser C, Garnett GP (2005) Host immunity and synchronized epidemics of syphilis across the United States. *Nature* 433:417–421.
5. Reef SE, Redd SB, Abernathy E, Zimmerman L, Icenogle JP (2006) The epidemiological profile of rubella and congenital rubella syndrome in the United States, 1998–2004: The evidence for absence of endemic transmission. *Clin Infect Dis* 43(Suppl 3): S126–S132.
6. Fox JP, Elveback L, Scott W, Gatewood L, Ackerman E (1971) Herd immunity: Basic concept and relevance to public health immunization practices. *Am J Epidemiol* 94: 179–189.
7. Anderson RM, May RM (1985) Vaccination and herd immunity to infectious diseases. *Nature* 318:323–329.
8. Gubler DJ (1998) Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev* 11: 480–496.
9. WHO (2007) *Scientific Working Group Report on Dengue 2007* (World Health Organization, Geneva).
10. Nisalak A, et al. (2003) Serotype-specific dengue virus circulation and dengue disease in Bangkok, Thailand from 1973 to 1999. *Am J Trop Med Hyg* 68:191–202.
11. Sabin AB (1952) Research on dengue during World War II. *Am J Trop Med Hyg* 1: 30–50.
12. Halstead SB, Simasthien P (1970) Observations related to the pathogenesis of dengue hemorrhagic fever. II. Antigenic and biologic properties of dengue viruses and their association with disease response in the host. *Yale J Biol Med* 42:276–292.
13. Burke DS, Nisalak A, Johnson DE, Scott RM (1988) A prospective study of dengue infections in Bangkok. *Am J Trop Med Hyg* 38:172–180.
14. Mammen MP, et al. (2008) Spatial and temporal clustering of dengue virus transmission in Thai villages. *PLoS Med* 5:e205.
15. Rabaa MA, et al. (2010) Phylogeography of recently emerged DENV-2 in southern Viet Nam. *PLoS Negl Trop Dis* 4:e766.
16. Raghwani J, et al. (2011) Endemic dengue associated with the co-circulation of multiple viral lineages and localized density-dependent transmission. *PLoS Pathog* 7:e1002064.
17. Ripley BD (1976) The second-order analysis of stationary point processes. *J Appl Probab* 13:255–266.
18. Gatrell AC, Bailey TC, Diggle PJ, Rowlingson BS (1996) Spatial point pattern analysis and its application in geographical epidemiology. *Transactions of the Institute of British Geographers* 21:256–274.
19. Diggle PJ, Chetwynd AG, Häggkvist R, Morris SE (1995) Second-order analysis of space-time clustering. *Stat Methods Med Res* 4:124–136.
20. French NP, McCarthy HE, Diggle PJ, Proudman CJ (2005) Clustering of equine grass sickness cases in the United Kingdom: A study considering the effect of position-dependent reporting on the space-time K-function. *Epidemiol Infect* 133:343–348.
21. Lynch HJ, Moorcroft PR (2008) A spatiotemporal Ripley's K-function to analyze interactions between spruce budworm and fire in British Columbia, Canada. *Can J For Res* 38:3112–3119.
22. Schreiber MJ, et al. (2009) Genomic epidemiology of a dengue virus epidemic in urban Singapore. *J Virol* 83:4163–4173.
23. Balmaseda A, et al. (2010) Trends in patterns of dengue transmission over 4 years in a pediatric cohort study in Nicaragua. *J Infect Dis* 201:5–14.
24. Waterman SH, et al. (1985) Dengue transmission in two Puerto Rican communities in 1982. *Am J Trop Med Hyg* 34:625–632.
25. Siqueira JB, et al. (2004) Household survey of dengue infection in central Brazil: Spatial point pattern analysis and risk factors assessment. *Am J Trop Med Hyg* 71: 646–651.