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Dengue diversity across spatial and temporal scales: local structure and the impact of host population size

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Abstract

A fundamental mystery for dengue and other infectious pathogens is how observed patterns of cases relate to actual chains of individual transmission events. These pathways are intimately tied to how strains interact and compete across spatial scales. Phylogeographic methods have been used to characterize pathogen dispersal at global and regional scales, but have yielded few insights into the local spatio-temporal structure of endemic transmission. Using geolocated genotype (N=800) and serotype (N=17,291) data, we show that in Bangkok, Thailand, 60% of cases living <200m apart come from the same transmission chain, versus 3% of cases separated by 1–5km. At distances <200m from a case (enclosing an average of 1,300 people in Bangkok) the effective number of chains is 1.7. This increases by 7-fold for each 10-fold increase in enclosed population, whether due to density or increased area; though increases in density over 7,000 people per km2 do not lead to additional chains. Within Thailand these chains quickly mix, and by the next dengue season viral lineages are no longer highly spatially structured within the country. In contrast, viral flow to neighboring countries is limited. These findings are consistent with local, density dependent transmission; and implicate densely populated communities as key sources of viral

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diversity with home location the focal point of transmission. These findings have important implications for targeted vector control and active surveillance.

Micro-scale transmission dynamics and the resulting competitive interactions between strains drive the distribution of infectious diseases in populations. Phylogeographic methods have been used to characterize pathogen dispersal at global and regional scales, but have yielded few insights into the local spatio-temporal structure of endemic transmission (1–6). Dengue virus is a mosquito-transmitted flavivirus grouped into four serotypes (DENV1-4). Dengue viruses infect over 300 million people annually, cause over 20,000 deaths and have circulated in Southeast Asia for decades (7, 8). Dengue's main vector, Aedes aegypti, has a limited flight range and often remains within the same household for long periods (9). Dispersal is driven by the complex interplay of the abundance of both vectors and humans, their movement and population immunity (10–12). The spatial scale of dispersal of dengue viruses may dictate the success of local control efforts. Introduction of novel variants to populations has been a prime determinant of burden for both dengue and other viruses (13–16) and thus understanding the processes that dictate viral dispersal is critical. The mystery of how dispersal proceeds requires linking patterns of disease incidence to actual pathways of transmission. Pathways of transmission are, in part, characterized by the number of independent transmission chains circulating in an area. For example the number of introduced versus locally acquired malaria cases has been used as a metric of endemicity (17).

Characterizing these pathways is a particular challenge for endemic pathogens, where overlapping transmission chains result in many unrelated cases appearing in the same communities at the same time, complicating efforts to understand how a pathogen is propagated and maintained. Surveillance systems typically capture a small fraction of infections (12, 18, 19); it has been estimated that only 12% of symptomatic dengue infections are captured in Thailand and up to three quarters of infections are asymptomatic (19, 20). In such settings, phylogenetic approaches can reveal information about the number of circulating chains and the relationship between chains at different spatiotemporal scales and allow us to investigate key unanswered questions in the epidemiology of endemic pathogens.

We sequenced the viruses of 640 geolocated dengue infections occurring from 1994–2010 from Bangkok and five other locations throughout Thailand (Figure 1A–B, Figures S1–S4, Tables S1–S3), and combined these with 160 GenBank sequences from elsewhere in Southeast Asia. In addition, we geolocated 17,291 hospitalized cases of dengue where the infecting serotype was known. Cases from Bangkok came from a children's hospital and had a median age of 8 years (interquartile range (IQR): 5–11). The cases from outside Bangkok came from tertiary care hospitals and had a median age of 10 years (IQR: 7–13). Approximately half the cases were female in both settings (Table S4). We developed two separate methods to estimate the number of circulating transmission chains (i.e., cases separated by a low number of intervening transmission events) at different spatial scales using sequence data and serotype data. We explore the effect of individual characteristics (age, sex) on the probability of observing cases from the same chain around the residence of

a case (21). In addition, we demonstrate how local population density plays a critical role in dictating the number of locally circulating chains and, using micro-simulation models, recreate the observed patterns. Finally we describe dengue's spread across spatial scales (neighbourhood, city, national and regional) both within a season and across seasons (where we consider pairs of cases with onset of symptoms within six months of each other to both come from the same season).

To determine the evolutionary time between each pair of viruses, we built serotype-specific time-resolved Bayesian phylogenetic trees (Figure 1C–F). We use a combination of bootstrapping observations and sampling trees from the posterior to capture sampling and tree uncertainty. Within Bangkok, we find a strong linear relationship between the evolutionary time between viruses and the spatial distance separating the homes of the cases from which they were isolated for up to 1.5 years (<27 transmission events) of evolutionary separation (Figure 1G). The median spatial distance between pairs of cases separated by under six months (<9 transmission events) was 670m (95% CI: 560–1,250).

Lineages appear to persist in the local vicinity of a case for up to six months. Homotypic (i.e., caused by the same serotype) cases with symptom onset within the same season living within 200m of each other have an 82% chance of having a Most Recent Common Ancestor (MRCA) in the prior 6 months (versus 46% in the prior 3 months, and 7% in the prior 6–24 months) (Figure S5). Cases living greater than 2km apart have a 1% chance of having an MRCA in the prior 6 months (versus 0.4% in the prior 3 months, and 6% in the prior 6–24 months). We therefore consider pairs of cases with symptom onset within the same season to be from the same transmission chain if their MRCA was within six months of the case with the earlier onset.

We find that 60% (95% CI: 33%–73%) of case pairs separated by <200m in Bangkok were from the same transmission chain, regardless of serotype. This decreases to 19% (95% CI: 11%–26%) for those <1km apart (Figure 2A). These results are robust to broader definitions of what constitutes a transmission chain (i.e., using different MRCA cut-offs, Figure S6). The rapid decrease in the probability of being part of the same chain by distance provides evidence for focal transmission, i.e., sequential transmissions are typically between households in the same neighborhood (22–24). This is consistent with empirical measurement of human movement that has found people spend most of their time within a few kilometers of their homes and the limited flight range of the vector (9, 25–27). While some infection events certainly occur far from home, the tight relationship between genetic and spatial distances suggests the majority of infection events occur near homes. This is further supported by a significant relationship with age, with the youngest, and presumably least mobile, individuals (those <5 years in age) having a 30% greater probability than older children (>10 years old) of being from the same chain as cases <500m from their home (95% CI: 16%–41%) (Figure 2D). Females were also slightly more likely to share a transmission chain with those nearby compared to males (Figure 2E). The apparent focal nature of transmission elucidates the mechanism by which increased susceptibility to severe disease following future infection with heterotypic serotypes might cluster spatially (23). If vaccination functions like a single dengue infection, the spatial scale of likely "priming"

infections could tell us where individuals who would receive benefit from vaccination are most likely to be located (though operationalizing such a strategy may be impractical) (28).

To extend our methods to settings where sequence data is unavailable, we developed a method to independently estimate the probability of pairs of cases being from the same transmission chain using only serotype data. We calculate the probability of cases being from the same chain as the excess probability of two cases occurring within some distance of each other during the same season being homotypic compared with the probability of two unrelated cases being homotypic (Figures 2A–B). Cases within a season are assumed to be unrelated if they are separated by >10km, the distance over which the probability of being homotypic remains constant (Figure 2C, Figure S7). The serotype-based analysis gives nearly identical results to the sequence-based analysis (Figures 2A–B).

We define the reciprocal of the probability that a pair of cases are from the same chain within a particular spatial distance as the effective number of transmission chains circulating within that distance. The effective number of chains represents a theoretical measure of the size of the pool of chains that any pair of cases within a given distance of each other are drawing from. For a sufficiently large population, this is a lower limit on the true number of chains within a particular distance of a case (see Materials and Methods for proof). In Bangkok, a mean of 1,300 people live within 200m of a case, and we find on average 1.7 (95% CI: 1.4–3.0) chains circulate in this population within a season. There is a linear relationship between log-population size and log effective number of chains (Figure 3A), with some deviation at small population sizes. In all of Bangkok, we estimate 160 (95% CI: 120–230) chains circulate within a season. While there exists a similar linear relationship between log-population size and log-effective number of chains, we find in provinces outside Bangkok host fewer chains during a season. A subset of Bangkok with population size equal to an outlying province will host 5.6-fold more chains (Figure 3A). This suggests that as rural communities become more connected we will see an increase in the number of chains.

There is substantial heterogeneity in the population density across Bangkok (Figure S8). We hypothesized that the number of chains within any location depends solely on the size of the local population, such that for areas of equal size, increasing population density results in additional chains. This hypothesis appears to hold up to a point. In Bangkok, at densities less than 7,000 per km2 the number of chains circulating in a population of a given size is the same regardless of the size of the area in which they live (Figure 3B–C). However, at population densities above 7,000 individuals per km2, the number of chains ceases to increase with population size. This is consistent with micro-simulation models of disease transmission that include local density-dependent transmission (i.e., increased transmissibility in denser areas), but not simulations with spatially random or local density-independent transmission (Figure 3D and Figure S9). Based on results from our simple modeling framework, we postulate that ecological interactions between virus strains in denser areas may limit the number of circulating strains. This could occur through competition for hosts mediated by immunity from previous infections or other mechanisms (e.g., vector avoidance after infection). In our density-dependent simulations, in areas of high population density previously infected individuals were 10-fold more likely to become

re-exposed than elsewhere (Figure S10). Competition between strains mediated through attempts to infect the same host or from strain-specific immunity from previous infections, means evolutionary pressures are likely to be strongest in these areas. High levels of asymptomatic disease and spatial heterogeneity in healthcare-seeking behavior mean we will only ever observe a small proportion of infections, and the proportion we observe may differ geographically (29). Using this modelling framework we demonstrate that our findings are robust to under-reporting and spatially-biased sampling (Figure S11). Further, we show that simulations cease to have spatial clustering consistent with our observations when a relatively small proportion (>10%) of infections occur away from home (Figure S12), strengthening the case for highly focal dengue transmission in and around homes.

To better understand broader geospatial dynamics of dengue, we compare the relative risk of infecting strains sharing an MRCA at specific time intervals for increasing spatial scales, from within Bangkok to across Southeast Asia. By only considering virus-pairs isolated within a short timeframe of each other (<6 months) in specific locations, we minimize the impact of spatial and temporal sampling biases that can affect phylogeographic analyses (Figures S13–S14) (30). Bangkok viruses isolated from individuals living <500m apart were 99 times (95% CI: 41–293) more likely to share a MRCA within 6 months of the earlier case compared to two Bangkok viruses isolated from cases >10km apart (distal Bangkok viruses) (Figure 4A). The probability of having a recent MRCA drops sharply as the spatial distance between Bangkok viruses increases. Viral diversity is reduced outside the capital with virus-pairs sharing an outlying province 19 times (95% CI: 2–73) more likely to have a recent MRCA than distal Bangkok viruses. Virus-pairs where one is in Bangkok and the other is in another province are 0.3 times as likely to have a recent MRCA compared to Bangkok distal viruses (95% CI: 0.1–1.4). However, after just a single season (i.e., MRCA between 6–24 months) these ratios approach one, suggesting viral lineages are well mixed across Thailand, both within Bangkok and between provinces (Figure 4B). The flow of virus across Thailand's borders appears to be much more limited. There are no virus-pairs in our dataset with an MRCA within 6 months of the earlier case when one virus comes from Thailand and another from elsewhere in Southeast Asia (i.e., Vietnam, Cambodia, Malaysia, Myanmar or Singapore). Even the probability of having an MRCA 2–5 years prior for Bangkok-Southeast Asia pairs is only 0.11 times that for distal Bangkok viruses (95% CI: 0.02–0.4). Overall virus-pairs were as likely to be from across countries (Bangkok-Southeast Asia pairs) as from distal parts of Bangkok only when separated by more than 8 years of evolutionary time (Figure S15) (findings are similar when using viruses from throughout Thailand, see Figure S16). These findings provide strong evidence that Thailand has endemic transmission that has limited connection to the rest of Southeast Asia. Thai borders may not be sufficiently porous to facilitate easy movement of virus. In addition, sick individuals may be less likely to travel internationally. Recent work has demonstrated high correlation in dengue incidence throughout the Southeast Asian region, with peaks during extreme climate years (31). Our findings support that ecological and environmental similarity rather than viral flow determines this synchrony.

By linking the distribution of case occurrence to the biological and ecological processes (transmission chains/competition) from which they arise, our work moves beyond previous findings that showed spatial clustering in dengue cases (23, 24, 32, 33). We also draw

connections between small-scale patterns and larger trends in dengue dispersal across the region. Further, we illustrate two independent, robust methods for revealing the spatial structure of transmission for endemic disease. Our findings that viral diversity increases with host population density supports a role for large urban settings as sources of a diverse set of viruses that could be dispersed elsewhere. The saturation of diversity at high host densities suggests that these dense areas may also be areas of intense competition between viruses, possibly contributing disproportionately to viral evolutionary pressures. For pathogen systems with multiple strains, our approach to estimating the number of circulating transmission chains within a community may provide a key surveillance tool for detecting changes in diversity accompanying expansion of particular types, characterizing differences in fitness between lineages or identifying populations that act as sources of viruses to other populations. Insights into microscale structure of endemic transmission are important for building policy relevant models of pathogen spread to appropriately target interventions. Our results illustrate that the structure of transmission is consistent with only certain assumptions about dengue transmission, and give empirical evidence of the importance of home location in dengue risk, supporting a role for targeted vector control around the residences of detected cases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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One Sentence Summary

Insight into dengue spread across spatial scales reveals local, density-dependent transmission in a season and longer-term endemic transmission countrywide

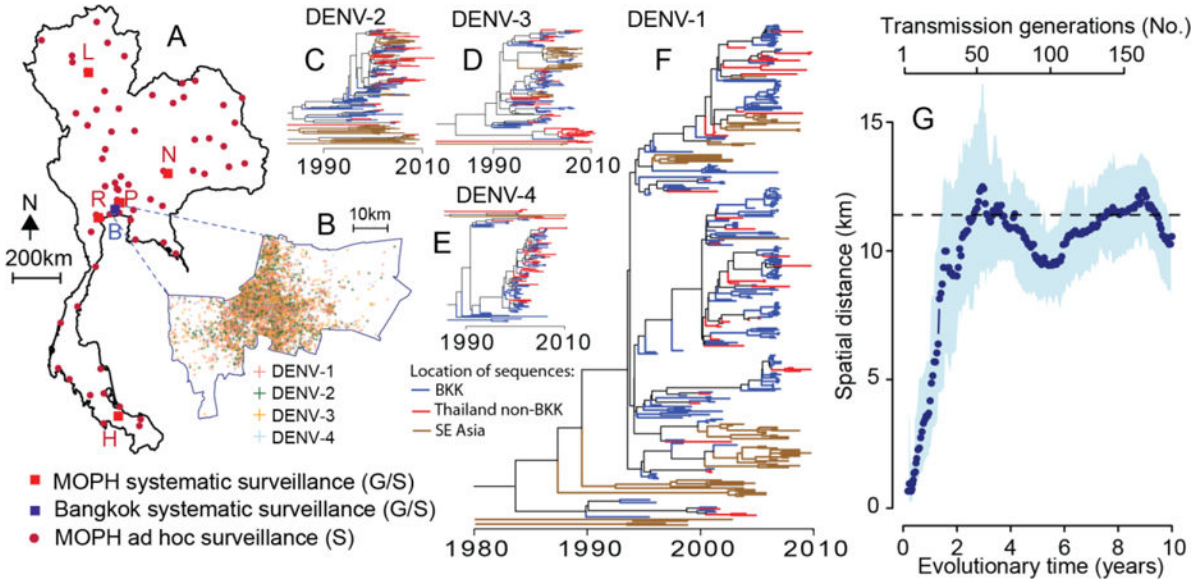

Figure 1.

(A) Map of Thailand showing location of case data (P-Pathum Thani, R-Ratchaburi, H–Hat Yai, L–Lampang, N-Nakhon Ratchesima, B-Bangkok). S–serotype data available, G–genotype data available. (B) Geolocated case data from Bangkok province. In total there were 7,511 DENV1, 4,265 DENV2, 3,371 DENV3 and 2,144 DENV4 cases. (C)–(F) Maximum Credibility Clade trees for DENV1 (N=306), DENV2 (N=210), DENV3 (N=157) and DENV4 (N=127). The colors of the tips represent the source of the virus. (G) Median spatial distance between virus-pairs from Bangkok separated by different total evolutionary times. The shaded area represents 95% confidence intervals. The number of transmission generations separating virus-pairs (top axis) is calculated by dividing the total evolutionary time by 20 days, the mean generation time for dengue.

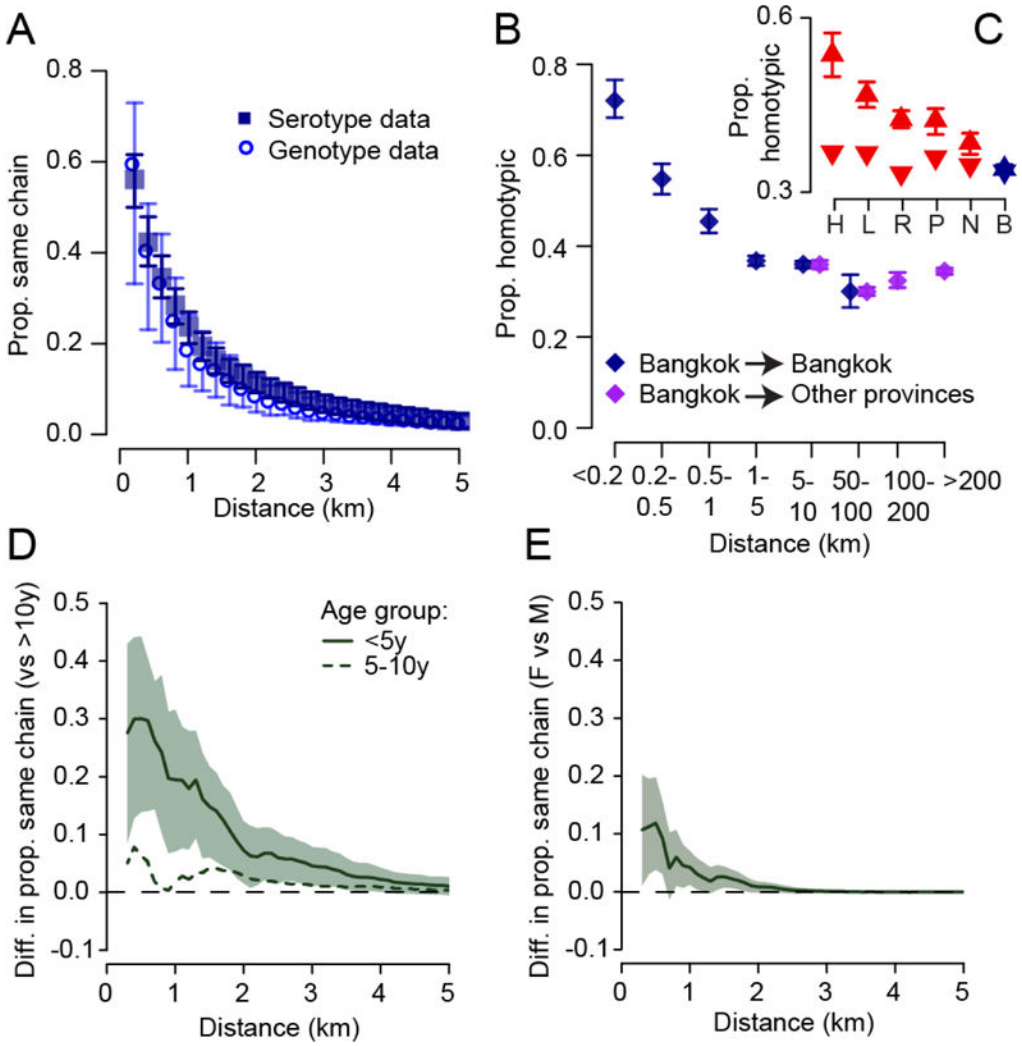

Figure 2.

(A) The proportion of case-pairs, sick within six months of each other that come from the same transmission chain when separated by different spatial distances within Bangkok. The estimates are calculated using either serotype (closed squares) or genotype (open circles) data (21). The error bars represent 95% confidence intervals. (B) The proportion of case-pairs, sick within six months of each other that are homotypic (caused by the same serotype) at different distance ranges where both cases are in Bangkok (blue) or when one is in Bangkok and the other is in another province (purple). The error bars represent 95% confidence intervals. (C) The proportion of case-pairs that are homotypic where both come from the same province (upwards facing triangle) and where they come from different provinces (downward facing triangle). The letters in panel (C) represent the provinces from Figure 1. The error bars represent 95% confidence intervals. (D) Difference in the probability that a case that is aged either <5y or 5–10y shares the same chain as another case within different spatial distances of their home versus the probability that a case that is aged >10y shares the same chain within that same distance. The shaded area represents 95% confidence intervals. (E) Difference in the probability that a female case shares the same

chain as another case within different spatial distances versus the probability that a male case shares the same chain within that same distance. The shaded area represents 95% confidence intervals.

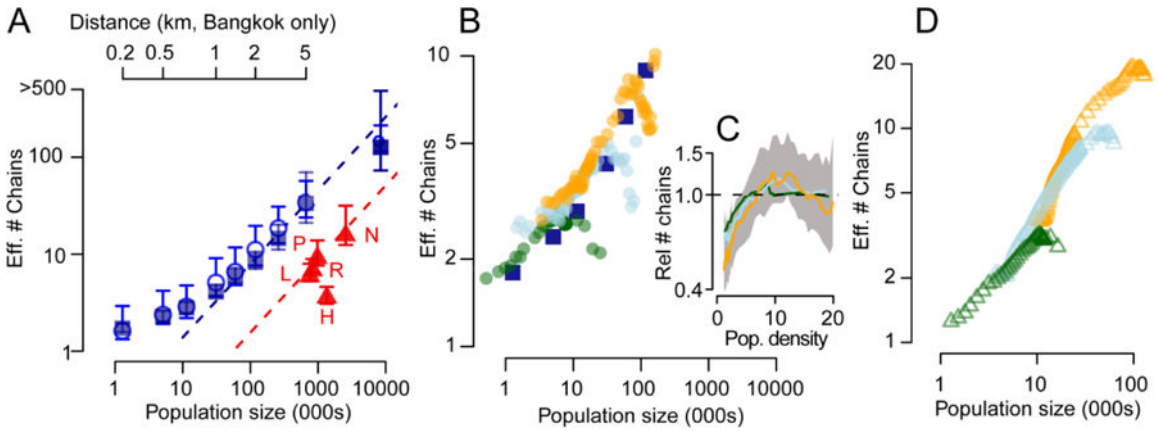

Figure 3.

(A) Number of discrete transmission chains circulating within a six-month period for different mean population sizes within Bangkok (blue) and across other provinces (red) calculated using either serotype (S) or genotype (G) data. Each intra-Bangkok estimate is the mean number of chains for different distances between cases (top axis). The mean population surrounding a case at that distance is on the bottom axis. (B) The number of transmission chains for fixed areas (radius of 0.5, 1 or 1.5km) with different population sizes within that area. The blue squares represent the mean number of chains within a fixed area across all population sizes from panel (A). (C) Number of chains at different population densities (in 000s/km2) relative to the expected number of chains irrespective of population density for different sized areas (the colors are consistent with panel (B)). The shaded area represents 95% confidence intervals for an area with radius 1.5km. (D) Number of transmission chains for different sized areas from simulations of density-dependent endemic transmission in a spatially heterogeneous population of 500,000 individuals where transmission occurs at <50m and is 2 times greater in the densest areas (population density of >20,000 individuals per km2) than elsewhere.

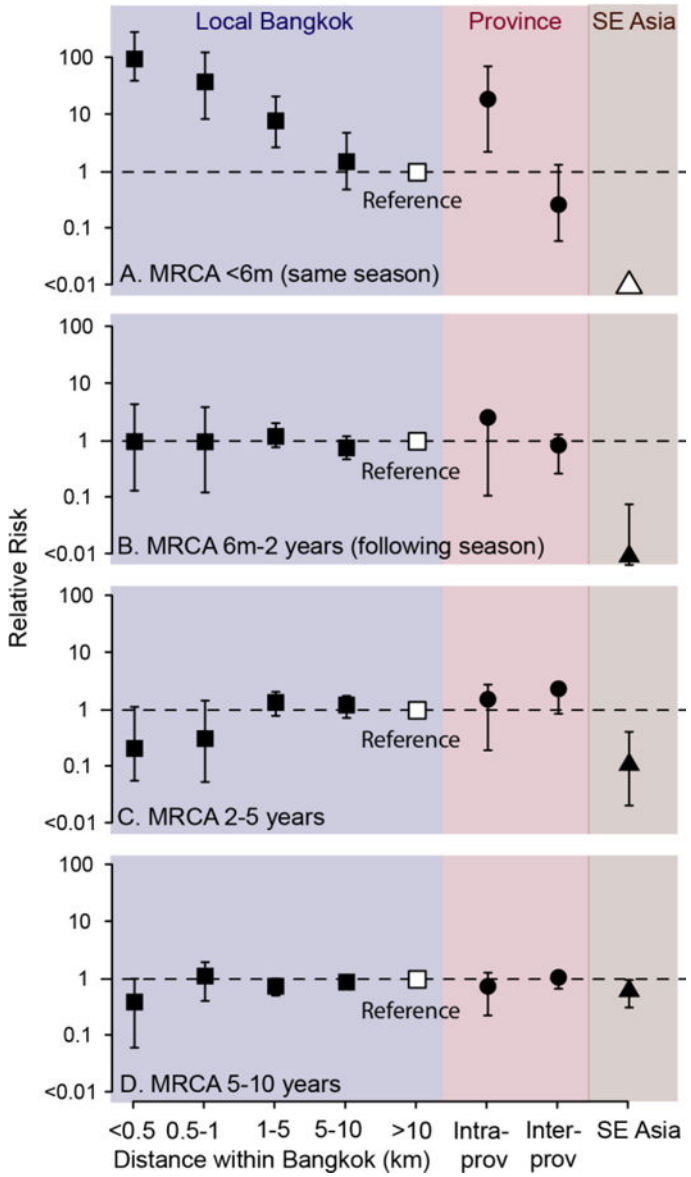

Figure 4.

Relative risk that a pair of viruses have an MRCA within a defined period. Each point represents the risk that a pair of viruses isolated from cases sick within six months of each other and living a particular spatial distance apart have an MRCA within a defined evolutionary timeframe (g1–g2) relative to the risk that a pair of distal Bangkok cases (defined as two cases from Bangkok separated by >10km) have an MRCA in the same g1-g2 range. Each panel represents a different g1–g2 range: (A) MRCA <6m (i.e., g1=0, g2=6m); (B) MRCA 6m–2y; (C) MRCA 2–5y; (D) MRCA 5–10y. 'Intra-prov' refers to pairs of viruses that both come from the same province outside Bangkok. 'Inter-prov' refers to cases where one virus comes from Bangkok and the other from a different province. 'SE Asia' refers to where one virus is from Bangkok and the other from another country in mainland

SE Asia (Vietnam, Malaysia, Singapore, Cambodia, Myanmar). The error bars represent 95% confidence intervals. The open triangle in panel A represents a value of 0.