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Commentary

Evolving virus plagues

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Recently, the AIDS pandemic and other new or emerging viruses have focused attention on emerging infectious diseases (1, 2). The factors involved in emergence are diverse and include global transportation, urban crowding and poverty, changing behavioral patterns, rapid virus evolution, human population growth, etc. Until now, no studies have clearly linked human population expansion with increasing rates of virus evolution.

The flaviviruses are an important genus containing more than 60 different viruses (3). Most of them are arboviruses (arthropod-borne viruses), but some are rodent and bat viruses. Among the major flavivirus agents of human disease are yellow fever virus, the dengue viruses types 1–4, Japanese encephalitis virus, West Nile virus, St. Louis encephalitis virus, and others. One human pathogen in the flavivirus genus which is not transmitted by insects or rodents/bats is hepatitis C virus, a blood-borne pathogen which afflicts 1–2% of the human population. The four types of dengue viruses have adapted very well to replication in humans during the last few centuries (3–5). Sporadic epidemics were first described about 200 years ago, but massive dengue fever epidemics have occurred since World War II, and dengue fever now involves many tens of millions of people annually. The virus replicates in monocytes and macrophages of humans and nonhuman primates to produce high blood levels of virus. These can infect *Aedes aegypti* or *Aedes albopictus* mosquito vectors taking blood meals from infected individuals, thereby maintaining human–mosquito transmission cycles. In tropical areas of West Africa and Asia, dengue virus is transmitted among nonhuman primates by mosquitos, but little is known about the role of such "forest cycles" in human outbreaks. It is clear, however, that most of the dengue epidemics which now sweep tropical areas of the world involve only human-to-mosquito-to-human cycles of transmission. These cycles are facilitated by population growth, rapid transportation, crowding into large urban centers, inadequate vector control, etc. (3–5). Obviously, these human epidemic cycles allow (indeed require) these viruses to adapt for efficient replication in both human hosts and mosquito vectors.

In contrast to these urban epidemics of dengue fever, the tick-borne flaviviruses of Europe and Asia are mainly zoonoses. Tick-borne encephalitis (TBE) viruses exist almost exclusively in "forest cycles" involving ticks and various vertebrate hosts. Humans play little if any role in these cycles, and human disease is accidental and dead-end (albeit sometimes severe). In work described in this issue of the *Proceedings* (pages 548–553), Zanotto *et al.* (6) have compared the molecular phylogenies of these flaviviruses and obtained remarkable results. They observed a continuous asymmetric phylogenetic tree branching among the tick-borne flaviviruses, as compared with an explosive radiation among the mosquito-borne flaviviruses for the last two centuries. The data in Fig. 3 are sobering. The number of dengue lineages has been increasing roughly in parallel with the increasing size of the human population over the last 200 years. As the human population (and the number of human dengue fever epidemics) have expanded, the opportunities for generation of new dengue virus lineages have grown apace.

The usual caveats must be applied to phylogenetic studies of this kind, but the data seem compelling. The flaviviruses are positive-sense RNA viruses, and like all RNA viruses, they have high mutation rates due to lack of efficient proofreading or mismatch repair systems (7, 8). However, the rates of evolution (mutation fixation) for the flaviviruses, the alphaviruses, and other arboviruses are generally lower by an order of magnitude or more than are the rates for many other (non-arthropod-borne) viruses (9, 10). This constraint on rates of arbovirus evolution has been ascribed to the need for arboviruses to maintain efficient replication capacity in the quite different selective environments imposed by arthropod vectors and vertebrate hosts (11). The two-phase lineages-through-time plot (LTTP) observed by Zanotto *et al.* for the mosquito-borne viruses is most reasonably explained by the likelihood that up until about 200 years ago most dengue epidemics were small and lineage extinctions were common. The authors discount the possibility that small sample size, sample bias, or choice of the E gene has affected the important inferences in this paper, and some completed polyprotein sequences confirm their E-gene results. A potential problem with all such analyses is the assumption of a somewhat constant molecular clock, rather than episodes of punctuated equilibrium. For example, the recent evolution of vesicular stomatitis virus in endemic foci in Central America shows more evidence for a geographical "clock" than for a temporal "clock" (12). Likewise, an earlier paper by Zanotto *et al.* (13) showed a strong correlation between the geographical and genetic distances of TBE viruses. Also, the rates of evolution of eastern equine encephalitis virus in North America have apparently increased by over an order of magnitude beginning in the early 1970s (10). Nevertheless, all RNA viruses can evolve quite rapidly and inexorably (7, 8, 14), and the inferences derived by Zanotto *et al.* (6) from the TBE and mosquito-borne virus lineage comparisons seem inescapable. The recent massive growth in epidemic spread of human dengue fever and the explosive radiation of dengue virus lineages are related.

Other evolutionary factors involved in the explosive radiation of the mosquito-borne flaviviruses remain largely unexplored. Despite their high mutation rates, RNA virus populations can often exhibit remarkable evolutionary stasis. Zanotto *et al.* (6) favor purifying selection over immune selection, but evolutionary divergence might also be promoted by other factors such as genetic bottlenecks (15, 16) or selection during replicative competition in large quasispecies populations (17). Regardless of mechanisms, these findings are cause for concern. As Monath (4) has pointed out, the tropism of dengue viruses for monocytes/macrophages and the related ability to replicate to very high levels in human blood raise some unsettling possibilities. New dengue virus serotypes (in addition to the four now circulating) will inevitably arise in future years. This will in turn lead to significant increases in dengue hemorrhagic fever and dengue shock syndrome—severe, life-threatening forms of dengue fever. These depend on prior antigenic sensitization (without significant immunization) by prior infection with a different serotype. Likewise, Monath (4) has suggested that dengue virus variants with altered tropisms might emerge during one of the nearly one hundred million human infections occurring each year. Although dengue viruses causing encephalitis or hepatitis or direct hemorrhagic fevers (without need for prior heterotypic infec-

tion) would seem to be highly unlikely, the probability is not zero. Similarly, the possible emergence of dengue virus strains capable of respiratory droplet transmission or efficient sexual/blood-borne transmission also cannot be assigned zero probability.

One must ask whether rapid evolution of other viruses might also be promoted by the burgeoning human population. The answer, of course, is yes. The elegant studies of Webster and his colleagues (18, 19) of the evolution of the influenza A viruses provide one example. These viruses generally exhibit relative evolutionary stasis in their avian hosts but rapid radiation while moving among human hosts. Human immunodeficiency virus type 1 is another obvious example of RNA virus quasispecies adapting to, and rapidly evolving within, numerous human hosts (20, 21). Finally, hepatitis C virus is another explosively evolving RNA virus, currently involving 1–2% of humans and causing a high incidence of persistent infection leading to cirrhosis or hepatocellular carcinoma (22, 23). In the past, this flavivirus quite likely evolved from an arthropod-borne virus which shed its requirement for insect vectors. As Zanotto *et al.* (6) point out, it is important to better understand patterns of arbovirus dispersal and evolution. It is also important to exert greater efforts to control the ever-expanding dispersal and evolutionary divergence of viruses such as dengue viruses.

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