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Genetics: A New Landscape for Medical Geography

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Abstract

The emergence and re-emergence of human pathogens resistant to medical treatment will present a challenge to the international public health community in the coming decades. Geography is uniquely positioned to examine the progressive evolution of pathogens across space and through time, and to link molecular change to interactions between population and environmental drivers. Landscape as an organizing principle for the integration of natural and cultural forces has a long history in geography, and, more specifically, in medical geography. Here, we explore the role of landscape in medical geography, the emergent field of landscape genetics, and the great potential that exists in the combination of these two disciplines. We argue that landscape genetics can enhance medical geographic studies of local-level disease environments with quantitative tests of how human-environment interactions influence pathogenic characteristics. In turn, such analyses can expand theories of disease diffusion to the molecular scale and distinguish the important factors in ecologies of disease that drive genetic change of pathogens.

Keywords

medical geography; disease ecology; landscape genetics; pathogenic evolution

Introduction

The history of geography is a history of an attempt to understand nature and society as mutually interacting forces. The idea of landscape, encompassing a multitude of definitions, has been used to combine nature and society into one intelligible entity. Hartshorne in the 1930s writes of the difficulties of geographers in defining landscape, and particularly in distinguishing any definition of landscape from that of area or region. His solution is to define landscape as the external form of the earth's surface, excluding the atmosphere but including human made objects, and to designate the material characteristics of this surface as landscape cover (Hartshorne 1939). Cosgrove's history of landscape indicates that landscape has evolved over time from a "way of seeing" to the study of the visible forms of an area to the experiential and dynamic interactions between humans and their environments (Cosgrove 1985). Landscape to geographers can be used to explore "the connections between geography and the humanities", and landscape, either physical or representational, is a reflection of culture in place (Cosgrove 1983; Agnew, Livingstone, and Rogers 1996). Landscape is also a geographic association between physical and cultural forms, or a place where a variety of natural realms shape, and are shaped by, daily human lives (Sauer 1925; Cosgrove 1983). The utility and role in geography of the concept of landscape continues to be examined, as witnessed by the panel discussion at the 2012 annual meeting of the

Association of American Geographers of the potential of landscape as a framework for human-environment research.

A recent extension of the scope of the work done in the social sciences is into the realm of genetics. The genetic character of individuals have been used in sociology to understand social structure and happiness, in anthropology to understand human migration and evolution, and in political science to understand voter participation or political persuasions (Rogers 1995; Cavalli-Sforza and Feldman 2003; Alford, Funk, and Hibbing 2005; Forster and Matsumura 2005; Bearman 2008; Fowler, Baker, and Dawes 2008; Schnittker 2008). Within geography, the collaboration with genetics has been more limited. While a genetic landscape has been explored for plants and animals by physical geographers, the focus has not been on human or human pathogen genetics as it has in the other social sciences (Riddle et al. 2008). For medical geographers, however, the genetic character of human diseases holds great potential for answering questions about how nature and society interact within a landscape to produce patterns of human health.

John Hunter's "challenge" to medical geographers was that all geographers could apply themselves to health problems of one sort or another, and that medical geography was thus situated "in the very heart or mainstream of the discipline" (Hunter 1974). We extend Hunter's challenge to the study of health genetics, and argue that we as geographers have a wealth of theory and methodology to lend to the increasingly important study of the evolution of infectious diseases. Geography is uniquely positioned to bridge the gap between the medical, social and natural sciences, and extending the traditional foci of medical geography, disease ecology and human-natural landscapes to encompass the genetics of human pathogens is imperative. Our challenge to medical geographers is to think beyond traditional disease patterns to explore patterns *within* disease, to those varying molecular characteristics of pathogens that are increasingly important in determining the range of disease diffusion and the efficacy of public health responses. This paper describes how geographers, by linking molecular analysis of pathogens to analysis of interactions between population and environment, can study the evolution of human disease-causing pathogens across space and through time. As emerging and re-emerging infectious diseases pose serious threats to global public health, understanding the influence of landscape processes on pathogen dynamics is vitally important.

Background

History of landscape in medical geography

Landscape science in the Russian school of geography, concerned particularly with the biophysical features of the environment, was an early influence on medical geography (Shaw and Oldfield 2007). The Russian parasitologist and geographer, Eugene N. Pavlovsky, used the phrase landscape epidemiology to describe the study of connections between disease nidi and their definitive geographic landscapes (Pavlovsky 1966). The natural nidus of disease to Pavlovsky was an area in the landscape where continuous circulation of a disease occurs due to the presence of hosts, vectors and agents. The empirical basis of Pavlovsky's nidi and landscape epidemiology were rodent burrows of the Asian steppe where plague circulated among rodents and fleas. Pavlovsky and his colleagues showed that this transmission cycle was as likely to be influenced by the environmental conditions of the burrows and surrounding steppe, the slope, altitude, drainage, soil quality, vegetation, etc. as it was by the characteristics of the rodents and fleas themselves. Just as the role of landscape in geography at large took a social turn in the 20th century, so too did the role of landscape in medical geography. As Meade puts it, "landscape epidemiology in a cultural landscape becomes resonant with the ideas of Jacques May (Meade 1977)." Jacques May, the "father" of American medical geography, was a French doctor whose experiences

in Indochina at the beginning of the 20th century informed much of his geographic understanding of disease. To May, disease was a “multifactorial phenomenon which occurs only if factors coincide in space and time (May 1950; May 1958).” Medical geography to May necessarily encompassed the geographical environments of these factors, since agents, reservoirs and hosts all varied geographically and were affected by the environment. It also encompasses the cultural landscape, since cultural choices such as house type, diet, crop preference or clothing could buffer or expose populations to disease factors. J. Ralph Audy, a medical doctor, speaks of medical ecology in relation to medical geography, arguing that the establishment, spread and evolution of infections is related to both behavior and environment, the social and the natural in combination (Audy 1958). People are organized in communities, as non-human species are in ecology, and comparing health outcomes across communities can illuminate the differential impacts of vegetation, physical barriers to disease spread, institutional processes and other compositional factors (Scott, Robbins, and Comrie 2012).

Landscape in geographic studies was heavily influenced by the work of French geographers, such as Vidal de la Blache, and there is evidence for the influence of French geographers on medical geography as well (Vidal de La Blache, P. 1903, Vidal de la Blache, P. 1922). Sorre, in 1933, realized that diseases were affected by both the physical and social geography of places, and that humans alter the natural environment in ways which affect disease patterns. Linking biogeography with human geography would allow medical geographers to explore the “interdependence of humans with their biogeographic environment” in the context of disease (Sorre 1933; Barrett 2002). Just such a link is established in the geographic field of disease ecology.

Disease ecology

The foundational idea of disease ecology is that human life is a process, a continual interaction between the internal and external environments (Dubos 1987). Disease ecology emerged in the second half of the 20th century, in reaction to the belief in medicine that infectious diseases were a thing of the past, and that curing disease was simply a matter of prescribing the right medication. Disease ecologists realized that diseases do not exist independently of environments or hosts, so “for adequate health maintenance, a vision broader than symptomology is necessary.” (Hunter 1974) To understand a disease, you must understand both the *person* and the *place* in which the infection occurs (May 1958; Hunter 1974; Meade and Emch 2010). Disease ecology thus concerns itself with “the ways human behavior, in its cultural and socioeconomic context, interacts with environmental conditions to produce or prevent disease.” (Meade and Emch 2010) These interactions are not static, however, but are dynamic and responsive to disturbance. As Dubos puts it, “nature” is not a constant entity, but is rather a passing place which organisms have adapted to, but places change and humans change (Dubos 1987). Human hosts, pathogens and insect vectors are all constantly adapting to new and changing conditions, developing resistance to drugs, buffers to disease exposure, etc., whether consciously or unconsciously. Disturbance in the ecological equilibrium of behavior and environment, via climate change, population growth, urbanization, agriculture, migration, etc., can have positive or negative effects on disease experiences, either magnifying or minimizing risk and exposure (Dubos 1965). Disease ecologists do not view humans as passive members of the disease system, however, but recognize that humans can adapt their behaviors or modify their environments in reaction to changes elsewhere in the system (Mayer 2000). Thus, disease ecology is inherently focused on integrating both the physical (environmental) and social aspects of human lives into an understanding of ill-health (Mayer and Meade 1994).

Although recognizing that human disease is the outcome of a complex and dynamic interaction between the internal and external environments of an individual or a population seems relatively straightforward, conceptualizing and understanding these interactions can be difficult. One way of doing so is to view disease at the intersection of three types of variables, *population*, *environment* and *behavior* (Meade 1977) (Figure 1). Population variables in this framework are those that affect individuals' responses to disease as biological beings, such as nutritional and immunological status, age, etc. The environment category encompasses all aspects of the built, natural and social environments that can affect disease outcomes. Behavior factors include both observable aspects of actions and culture, such as social organization, technology, diet, etc., as well as less tangible variables like perceptions of risk. Disease outcomes are the result of place and time specific interactions among these variables. To understand H5N1 avian influenza in Vietnam, for instance, relevant interacting population, environment and behavior variables are shown in Figure 2. Environmental conditions that favor influenza transmission, such as water bodies, combine with human population density patterns and behaviors such as raising backyard poultry flocks, and population characteristics like avian species-specific immune responses to influenza to produce differing spaces of influenza risk and transmission.

Understanding such place and time specific interactions and adaptations as they relate to disease is a strong tradition in medical geography. The mobility of humans, of insect and animal vectors and of pathogens influences observable patterns of disease outcomes within a landscape. Roundy (Roundy 1978) describes the importance of human mobility in determining exposure to pathogens or the introduction of pathogens from one location to another, i.e. disease diffusion. Meade (Meade 1977) explored the differential effects of time spent in risky habitats on disease outcomes of sub-populations, showing that men, women and children experience disease differently based upon their daily environmental interactions. And Prothero (1961; 1963) detailed the ways in which human mobility in Africa challenged efforts to control disease such as malaria and trypanosomiasis, wherein nomadic animal herding or seasonal employment in mining or agriculture diffused and re-diffused infection from one area's insect vectors to another. An understanding of the impacts of circulation and mobility of humans and their associated diseases is increasingly important as populations move through what Zelinsky described as a mobility transition, with daily distances traveled and international labor movement rapidly expanding (Zelinsky 1971).

In his "Challenge to Medical Geography," John Hunter advocated that disease ecology research should not end with cartography (1974). Rather, the genesis and manifestation of disease must be explored; medical geographers should not stop at describing pattern but push further to explore process. Considering the genetic character of disease, in addition to places of presence or absence, lends itself to this deeper understanding of processes that produce patterns. Landscape variation does not simply drive variation in disease outcomes, it also creates differential rates of change in pathogens across space and time, and differential patterns of molecular characteristics such as drug resistance.

Landscape ecology

Hunter's call for pattern *and* process in medical geography is one which is echoed in another field, landscape ecology. Landscape ecology, while explicitly spatial, emerged in ecology rather than geography in large part because of the loss of human-environment interactions as a focus for geographers in the mid-twentieth century (Wiens et al. 2007). While medical geography and disease ecology were still inherently concerned with humans in the environment, the focus for much of geography was on environment only as it related to human activity, i.e. location and distribution of economic and social geography.

As in geography, the term landscape has multiple definitions for landscape ecologists. To Turner, landscape is an area where at least one factor (species) of interest is spatially heterogeneous (Turner 2005). Within this spatially heterogeneous landscape, the relationships between biotic and abiotic elements drive observed patterns. To Naveh, a landscape is a selforganizing system with humans as participants in the natural environment (2007). Others consider landscape as a mosaic, made up of patches of interacting species of varying sizes, and that the hallmark of a landscape is variation in patterns of species and behaviors generated by processes, such as migration, operating at a variety of spatiotemporal scales (Urban, O'Neill, and Shugart Jr. 1987). Landscape is one level in a hierarchy of scales, from individual to region and globe, and is influenced by processes both up and down the spatial scale. Landscape ecology, then, is the study of the reciprocal interactions between ecological processes and spatial patterns (Turner 2005). In recent years, just as the social sciences have embraced genetics as a new field of inquiry, so too has landscape ecology in the form of landscape genetics. For landscape ecologists, genetic information from species allows questions about process, e.g. migration and adaptation, to be understood from molecular spatial patterns.

Landscape genetics

The emerging interdisciplinary field of landscape genetics is based on the idea that exploring spatial variation in genetics can illuminate how organisms exist in and move across the landscape. Landscape genetics combines theory and methods from population genetics and landscape ecology in order to explore interactions between evolutionary processes and environmental features (Manel et al. 2003; Guillot et al. 2005; Holderegger, Kamm, and Gugerli 2006; Storfer et al. 2007; Balkenhol et al. 2009). Landscape genetic studies differ from those of biogeography and phylogeography in that they operate at finer spatial and temporal scales, and are made possible by the convergence in the past decade of publicly available, high-resolution molecular and geospatial datasets (Storfer et al. 2007). This new data availability allows genetic outcomes to be linked to the associated population and environmental character of their places of incidence.

There are two steps to a landscape genetics analysis. The first is to identify how genetic characteristics vary in space, the second is to correlate those patterns with specific characteristics of the underlying landscape (Manel et al. 2003). The strength of landscape genetics is that it is based upon real-world linkages between genetic outcomes and landscapes, rather than abstracted modeling methods that focus more on genetics than landscape (Holderegger and Wagner 2006). Identifying the real-world factors that drive evolutionary processes in turn enables scientists to predict future developments of genetic diversity, particularly important given the emergence of new pathogens or drug resistant pathogens (Guillot et al. 2005).

Landscape genetics draws upon landscape ecology methods for analysis, while using data from population genetics as the outcome variable of interest. Landscape genetic studies have, so far, been confined primarily to the study of plants and animals rather than pathogens or people. Ecologists and biologists have used landscape genetics to infer colonization patterns, disease response, habitat restriction and extinction events of mammals, reptiles, insects and plants (Piertney et al. 1998; Sork et al. 1999; Mock et al. 2007; Wheeler and Waller 2008). In the past few years, however, there has been recognition by disease ecologists that landscape genetic techniques can be used to explore drivers of disease spread and parasite transmission as they relate to human illness (Archie, Luikart, and Ezenwa 2009; Sloan et al. 2009; Biek and Real 2010; Criscione et al. 2010). As yet, little work has applied landscape genetic techniques to pathogens that are anthrozoönotic, infecting both humans and animals. Some very early work on typhus rickettsia in the mid-twentieth century by medical geographers and others indicated that geographically distinct

subspecies evolved in relative isolation, limited in their dispersal by oceans and deserts (Audy 1958). More recently, research into malaria has shown distinct geographical distributions among genetic lineages of drug resistant *Plasmodium falciparum* parasites in Africa, suggesting drug resistance dispersal patterns and potentially different responses to treatment based on genetic origin (Pearce et al. 2009). Analysis of schistosome fluke genetics in Kenya indicates that boundaries between bodies of water such as lakes and streams restricts the exchange of genetic material among the worms, which in turn would influence genetic patterns among infected humans (Steinauer, Blouin, and Criscione 2010).

Such applications, however, tend to focus on the genetics of pathogens but not the implications of those genetic patterns within human hosts or the population-environment interactions which mediate the host/pathogen interface. Landscape genetics research of human pathogens needs to be informed by knowledge of how such pathogens are influenced by human and animal interactions with environments, and how such interactions vary in space and time, in order for research findings to be valid or valuable for public health efforts. The strength of disease ecology as an integrative science, drawing upon knowledge about human-environment interactions, lends itself to the application of landscape genetic techniques, developed for plant and animal studies, to the study of pathogenic evolution.

Merging disease ecology and landscape genetics: a new avenue for the investigation of human disease

There is much to be gained from landscape genetic analysis informed by medical geography's disease ecology. It is now possible to push beyond traditional presence/absence disease outcomes to study the changing character of the pathogens themselves to answer questions about what features of human-natural landscapes drive disease emergence and pathogenic evolution. Questions that the integration of landscape genetics and disease ecology can answer include: What local level population and environment variables are related to molecular evolution of pathogens? What behavioral or natural environmental features act as barriers to the spread of drug resistant pathogens? What population interactions with the environment drive diffusion of new disease variants across a landscape? How do spatially variable interventions such as vaccination or vector eradication impact pathogen genetic distributions?

Infectious disease is not a static outcome, but rather a demonstration of the relative success of a living and evolving organism in making a human a host. From a medical geographic perspective, disease is the result of a maladaptation between humans and their environment. Thus, linking medical geography and landscape genetics gives a view that spatial patterns of disease within a landscape can reveal the interacting population and environmental processes allowing disease to persist and/or evolve. Concomitantly, it can also reveal landscapes where diseases cannot persist, giving insight into human-environmental interactions beneficial to human hosts and detrimental to pathogenic success. As Mayer, a medical geographer writes, the emergence and reemergence of infectious diseases in the world is as much the result social/ecological/geographical changes as it is molecular evolution of pathogens, and the geography of genetic mutation matters (Mayer 2000). Using the former, social/ecological/geographical, to understand the latter, molecular evolution, is now possible as the result of the increasing availability of spatially referenced genetic data and high resolution natural and social spatial data.

Genetic Data Sources and Molecular Measurement Methodologies

Genetic data are available from multiple sources, including nasal swabs of sick animals or humans or dried blood spots taken during health surveillance. Using such datasets, there are then multiple measures of genetic characteristics available for use in a landscape genetics/

disease ecology study. Perhaps the simplest is a binary indicator variable, used to show whether a virus or parasite found in a human or animal host had (a) specific mutation(s) or not. The presence/absence of amantadine drug resistance among H1N1 viruses, for instance, can then be related to background population and environmental variables associated with the locations where those viruses were isolated.

More complex measures of the genetic characteristics of human pathogens take several forms, and this form is typically dependent on the nature of the pathogen itself. Viruses, for instance, can have their full genetic sequences coded as a text string of As, Ts, Gs and Cs (Adenine, Thymine, Guanine, Cytosine) and then these text strings can be used to determine the amount of genetic difference that exists between two viruses. Distance measures of this sort are frequently used in H1N1, H5N1 and other influenza studies (Wan et al. 2008; Carrel et al. 2010). Such differences can further be visualized in a phylogenetic tree, indicating not just the amount of genetic change that has occurred between the isolation of two separate viruses, but also the evolutionary lineage of such viruses. Viruses are often categorized according to family groupings known as clades, with clades representing moments in space and time when viruses have taken new evolutionary paths. Exploring what drives the emergence of such clades, or what influences the genetic distance between viruses on different branches of a phylogenetic tree, can help to illuminate host-pathogen-landscape interactions.

Another popular method for measuring genetic relatedness or difference is the Fixation Index (F_{ST}). The F_{ST} is used to determine the differences in allelic frequencies within and between populations (Wright 1965). An allelic frequency indicates how often a specific genetic variant exists in an individual or population. For instance, in humans at a global scale it has been applied to the study of chromosomal differences among regions and continents in an effort to infer historical population dynamics (Li et al. 2008). In disease studies the F_{ST} has been used to explore variation in HIV genetic sequences across the U.S.-Mexico border, as well as to inform the spatial structure of malaria elimination campaigns in the Comoros Islands (Mehta et al. 2010; Rebaudet et al. 2010).

The application of disease ecology theory to landscape genetic methodologies is highly dependent on the availability of georeferenced genetic data. As the utility and popularity of spatial analysis of disease has increased over the last several years, so too has the collection of spatial attribute data associated with disease locations and genetic outcomes. The scale at which such spatial data are available, however, often limits the types of analysis conducted. For some diseases, particularly those with high levels of stigma, spatial attribution is limited by ethical and privacy issues. For example, releasing HIV genetic sequences geocoded to the address of individuals is ideal for answering questions of how transmitted drug resistance moves through a population but studies are complicated by serious questions about the privacy of individuals and the protection of their rights. For other diseases with less stigma, such as *E. coli* infection, the issue is that the spatial data associated with genetic characteristics of isolates is at a spatial scale which is too large for meaningful analysis of the ecology of disease genetics. Even on GenBank, the open-access host for millions of genetic records for infectious diseases detected worldwide, the location associated with the majority of records are at the county, district, province or state level. This leads to the assigning of multiple genetic sequences to the centroid of one areal unit, and complicates the association of those genetic sequences with background population and environmental drivers.

The analytic limitations of suboptimal spatial attributes for genetic data can thus restrict the utility of the findings. For geographers interested in the diffusion of drug resistance among pathogens across a landscape, the precise, or nearly precise, locations where drug resistant

pathogens were found is important. Increasing the resolution at which a disease is studied, to the genetic scale rather than the host population scale, has incredible potential for building upon the disease diffusion work of earlier medical geographers, but it must be matched by an increasing resolution of spatial scale (Pyle 1969; Stock 1976; Cliff et al. 1981; Cliff, Haggett, and Graham 1983; Patterson and Pyle 1983; Cliff and Haggett 2004).

Human & Environment Data Sources

The linking factor between genetic characteristics of disease isolates and the population and environment characteristics of the places they are found is the geographic location of incidence. A geographic information system (GIS) can be used to overlay multiple layers of data to generate a profile of the landscape characteristics associated with places of disease incidence. The wide variety and high quality of spatial data available for free or fee-for-service online allows for the investigation of the molecular ecology of a diversity of diseases. For instance, livestock densities and farming classifications are available from the Food and Agriculture Organization. Global population densities are mapped by the Center for International Earth Science Information Network (CIESIN) (Center for International Earth Science Information Network (CIESIN) 2010; Hansen et al. 1998). CIESIN also distributes spatial data on flood hazards and rural/urban population distributions. The Global Landcover Facility makes freely available global data on land use/landcover, protected areas and water bodies (Hansen et al. 1998).

Many nations now make the data collected from annual or decennial censuses available online, allowing environmental features to be connected to population characteristics such as wealth, socioeconomic status, age distribution, race/ethnicity distribution, etc. Projects such as AfriPop and AsiaPop are focused on providing accurate and high resolution population distribution information, particularly for countries with unreliable or highly intermittent censuses (Tatem et al. 2007; Linard, Gilbert, and Tatem 2010). Cell phone records, GPS trackers and updates from mobile devices, such as Twitter usage and FourSquare checkins, can help to indicate patterns of human mobility and movement through risky landscapes (Vazquez-Prokopec et al. 2009; Le Menach et al. 2011; Noulas et al. 2012). Capturing this temporal variation in population distribution is important, given the potential for significant differences in human-environment interactions and opportunities for pathogenic diffusion or change based upon timing of measurement (Kwan 2012).

These spatial databases, or data sources such as mobile phone records whose potential has yet to be fully realized, are what enable future exploration of landscape drivers of genetic change, allowing researchers to connect population-environment data to the molecular characteristics of pathogens. Under a disease ecology framework, the ecology of the disease drives the variables of interest in the study. Vectored diseases are more likely to be influenced by environmental variables such as wind direction, altitude, and land cover than are direct contact diseases such as HIV.

Once genetic variation has been linked spatially to hypothesized population and environmental drivers of molecular evolution, there are a variety of methodologies available to quantify the influence of these drivers on genetic outcomes (Table 1). These methods make use of a variety of measures of genetic characteristics of pathogens (as described above) as their outcome variable of interest, and explore the varying importance of population and environmental features in a local-level landscape on those genetic characteristics. Barrier detection analysis, for instance, looks for spaces where sharp changes in genetic characteristics of pathogens occur, and then seeks to align those spaces with underlying breaks in landscape features such as rivers, mountains or unpopulated places. While there are ongoing developments in the methodologies available for landscape genetic studies, the majority of landscape genetic methods listed in Table 1 have a long history of

use in landscape ecology, population genetics, geography or public health, but are only now being applied to the study of the ecology of human pathogenic evolution. It is the theoretical connections between landscapes and pathogenic evolution that is a revelation and a revolution for disease ecology.

Empirical work combining disease ecology and landscape genetics

We used landscape genetics analysis to explore the evolution of highly pathogenic H5N1 avian influenza viruses in Vietnam. Although much scholarly attention has been paid to the phylogenetic character of H5N1 viruses, most of this work is either aspatial or considers geography in a descriptive sense or at a coarse spatial resolution, such as the nation (Smith et al. 2006; Janies et al. 2007; Small, Walker, and Tse 2007; Wallace et al. 2007; Duan et al. 2008; Dung Nguyen et al. 2008; Wang et al. 2008; Wallace and Fitch 2008; Zhao et al. 2008; Pfeiffer et al. 2009; Liang et al. 2010). Alternately, those studies that do incorporate human-environmental interactions into the study of H5N1 do not take into account the molecular characteristics of viruses (Pfeiffer et al. 2007; Small, Walker, and Tse 2007; Gilbert et al. 2008; Peterson and Williams 2008; Williams, Fasina, and Peterson 2008; Henning et al. 2009; Tiensin et al. 2009; Paul et al. 2010; Martin et al. 2011). We sought to address these gaps in the literature by integrating genetic, environmental and population variables to explain how genetic characteristics of viruses differ by species, where and when barriers slow H5N1 viral evolution, and how population and environmental characteristics interact to influence molecular change. Molecular change is important to consider in the case of H5N1, since viruses are constantly evolving and potentially developing the potential to pass easily from human host to human host. Central to these studies is the idea that human modification of natural environments for purposes of poultry production creates places in space and time that either positively or negatively influence the spread and evolution of avian diseases, including avian influenza. Only the unique application of landscape genetics methods informed by disease ecology theory to the study of an anthrozoönotic pathogen allows such an assessment.

The dataset for these analyses consists of 125 highly pathogenic H5N1 viruses isolated in Vietnam from 2003 to 2007. No viruses are from 2006, a year when no H5N1 was officially recorded in Vietnam as the result of an intense eradication campaign by the Vietnamese government. For each virus there is a full or nearly full genetic sequence, allowing for the calculation of genetic distance. Influenza viruses have eight gene segments, so for each virus there are eight genetic distances to consider (given that gene segments evolve at different rates from one another). Two types of genetic distances were calculated using phylogenetic trees, one considered pair-wise genetic distance between each of the 125 viruses and the other calculated tree branch lengths between the 125 viruses and a common progenitor virus. For each virus there was also information on the host species in which the virus was isolated (110 were found in ducks or chickens), the date of isolation and the province of isolation. This information was used to create geographic and temporal distance measures, as well as to geocode the viruses to their location of incidence.

The majority of rural Vietnamese raise backyard poultry flocks of chickens and ducks. Chickens are found in greater numbers in the north, around the Red River delta, while duck production is favored in the south in the Mekong River delta, although both types are found in both places. Chickens are typically confined to the household area, while ducks are often moved out of the household and into nearby fields and ponds to feed on insect pests, fallen rice seed and weeds. Ducks thus have greater opportunity to interact with either wild birds or ducks from other households, increasing potential for H5N1 viral transfer, whereas chickens are likely to be exposed only to infected household ducks or humans carrying the virus on their clothing or other surfaces. Cluster analysis and multiple analysis of variance (MANOVA) were used to determine whether these differing ecologies in animal husbandry,

and attendant differences in potential disease exposure, would be expressed in the genetic characteristics of viruses found in chickens versus ducks. Results indicate that duck viruses have greater amounts of genetic change than do chicken viruses, suggesting greater amounts of viral mixing and mutation have taken place (Carrel et al. 2011).

Viruses were clustered according to their genetic characteristics, such that viruses included in clusters were more genetically similar to one another than to viruses in other clusters. No spatial attributes of viruses were taken into account, cluster assignments were based solely on the genetic distances from an ancestral virus measured for the eight gene segments of the flu viruses. When these cluster assignments were mapped according to province of isolation and species of isolation (Figure 2), it became apparent that one cluster (#8) was made up of viruses found only in southern ducks. These southern duck viruses were genetically more distant, i.e. more evolved from an ancestral virus, than were other duck and chicken isolates. The average genetic distance on all eight gene segments, stratified by whether that virus was found in a chicken or a duck, indicates that duck viruses have higher amounts of genetic distance from the progenitor ancestral virus than do chicken viruses (Table 2). The increased genetic change associated with duck viruses was further evidenced in the multiple analysis of variance results (Table 3), which indicated significant variation between the genetic characteristics of viruses found in ducks as opposed to chickens. The important influence of temporal and genetic distance between isolates was also established in the MANOVA. What these findings suggest is that ducks are a species that should be carefully monitored for H5N1 avian influenza, and that regions with high duck population densities may be areas where new strains will emerge, potentially with human-human transmission capabilities. The relationship between ducks and increased risk of H5N1 incidence has been discussed by other researchers, but this landscape genetic work indicated that spaces where ducks are raised in high numbers, as they are in the Mekong Delta of Vietnam, are also spaces where high rates of genetic change in viruses can be expected (Gilbert et al. 2006; Tiensin et al. 2007)

The majority of H5N1 viral isolates in the dataset are found in the northern provinces around Hanoi and the southern provinces around Ho Chi Minh City, sites of high human and poultry population densities. This is due both to the prevailing patterns of H5N1 in Vietnam (with more in the north and the south) as well as to the particularities of H5N1 data collection and availability within Vietnam. Previous research had suggested an isolation by distance model of genetic movement across Vietnam, with viruses introduced into the north and later being found in the south, evolving as they move across the landscape (Carrel et al. 2010). There are significant changes in both the social and environmental landscape from northern to southern Vietnam, from differing human population densities in the Red River and Mekong River deltas versus the center of the country, to higher densities of chickens in northern Vietnam and higher densities of ducks in southern Vietnam, to climatic differences between the more temperate north and the subtropical south. These differences create potential barriers to diffusion. Principal components analysis (PCA), combined with spatial interpolation methods, and wombling of the genetic distance measures were used to determine whether the low human and poultry population densities in central Vietnam acted as a barrier to gene flow. PCA results exploring the structure of measures of genetic change for each of the viruses' eight gene segments (Figure 4) seemed to suggest that barriers to gene flow could exist, given the sharp changes within Factor 1 and 2 scores over small geographic distances. Wombling looks for steep zones of change in a genetic surface, premised on the idea that such zones are indicative of underlying barriers or boundaries to genetic exchange. Ultimately, the Wombling analysis did not support the initial suggestion from the PCA that boundaries to genetic exchange exist in Vietnam, and we found that while Vietnamese viruses do change between northern and southern Vietnam, there are few statistically significant barriers to gene flow, and those barriers that do exist are sporadic in

space and time (Carrel et al. 2012). In other words, despite the changing human and environment landscape across the north-south extent of the country, H5N1 seems to be able to move through these changing environments without facing barriers to incidence or evolution. These findings are important when considering the potential for intervention in H5N1 outbreaks in Vietnam, indicating that the government cannot rely on breaks in human and bird population density or temperature or other climatic variation to provide natural barriers to H5N1 viruses spreading and evolving, rather that viruses will be able to move easily across the length of the country unless specific actions such as bird culling or closing live bird markets are taken to prevent such diffusion.

A disease ecology framework was used to generate population and environment variables hypothesized to be related not only to H5N1 incidence but also to genetic change in viruses (Figure 3). Measures at the provincial level for a number of these variables were then generated and associated with each province's viral genetic characteristics in order to assess the influence of landscape characteristics on genetic change (Table 4).

Given the large number of dependent (8 genetic distances) and independent variables, non-metric multidimensional scaling (NMDS) was used to narrow the number of independent variables used in regression analysis to determine which landscape variables was associated with higher rates of viral evolution. NMDS methods can be very useful in a combined disease ecology/landscape genetics analysis, given the holistic nature of disease ecology and the inclusion of a large number of potentially relevant variables from behaviors and environments that can impact genetics. The three-dimensional NMDS of the 125 scaled H5N1 viruses (scaled according to each virus' 8 genetic distance measures, one for each gene segment) was associated with all the population and environment variables hypothesized to be related to genetic change (Table 4). Only those variables that had high correspondence with the scaling were retained. These variables were then used to create the initial regression model examining the influence of population and environment variables on the scaled H5N1 viruses. The final regression results (Table 5) suggest that it is a combination of environments suitable for host interaction and species mixing (i.e. water surface per province) as well as population characteristics (higher population density, educational and socioeconomic status) that create provinces with higher rates of H5N1 molecular evolution (Carrel et al. 2012). Areas with these characteristics should thus be carefully monitored for the appearance of potentially lethal H5N1 influenza viruses.

These studies suggest that understanding spatial patterns of genetics can illuminate not only the ecology of a disease and the progressive evolution of the causative pathogen, but also how that pathogen responds to its environment. Such work has implications for disease control efforts, indicating the types of landscapes where efforts should be focused to prevent not only incidence of H5N1, but also the spaces where new strains will be likely to emerge.

Conclusion

The majority of public health and medical geography studies of infectious disease treat the diseases themselves as static outcomes. The reality, however, is that pathogens are constantly evolving to better evade immune responses. This is particularly true for RNA viruses such as influenza and HIV which are showing increasing evidence of drug resistance. In landscape genetics, disease is treated as a continuous variable instead of dichotomous; not only does the disease exist in a place (or person), it has a particular genetic sequence that may or may not differ from an adjacent case. Ongoing work by the authors extends landscape genetic methods from viruses to other pathogenic agents, exploring the drivers of malaria drug resistance in the Democratic of Congo and the relationship between Methicillin-resistant *Staphylococcus aureus* and confined animal feeding operations in Iowa.

Using a hybrid conceptual approach combining disease ecology theory with population genetics measures and landscape ecology methods can allow medical geographers to understand how interactions in the landscape between humans and their environment act upon the evolution of any type of pathogen.

Our results for H5N1 influenza in Vietnam indicate that using landscape genetics methods can help us understand species differences in influenza evolution, the presence (and absence) to population or environmental barriers to gene flow, and how local-level environmental variables correlate with increased genetic change. While these findings are interesting, they are only the first step towards merging disease ecology and landscape genetics. The highly detailed genetic data, and relatively high resolution population and environment datasets, used in this research was unfortunately not matched by highly accurate location information for the influenza cases. Until the spatial location attribute data for H5N1 and other diseases matches the genetic and environment data in accuracy and precision, the findings of such research will be limited to a coarser scale. Future landscape genetics and medical geographic research also needs to better account for the temporal aspect of both genetic and environmental change, matching the timescale at which evolution occurs (and is being measured in population genetics) to the timescale at which landscape variables are measured.

Medical geographers are in the position to speak both to landscape ecologists and their ecological studies and to public health practitioners and their modeling strategies. A discipline is only as strong as its contributions to both theory and methods. Landscape genetics is an important and vital way for medical geographers to build upon and expand theories and descriptions by May, Hunter, Meade, and others of local-level disease environments with quantitative tests of how people interact with their environment in ways that affect disease dynamics. The results of spatial analysis of pathogenic evolution can then re-inform theories of disease diffusion and cultural ecologies of disease. As humans continuously modify their environments, via expansion of road networks, increased human mobility, changing water and hygiene interactions, population growth and migration, movement into urban areas, and extensification and intensification of agricultural production, the fieldwork of medical geographers working at multiple spatial scales and the subsequent understanding of both seen and unseen landscape level processes will be vital. As yet, much of the theory and methodologies that medical geographers bring to an understanding of both disease and human-environment interactions across space and time have not been applied to the study of pathogenic evolution, but the promise of such an application is immense.

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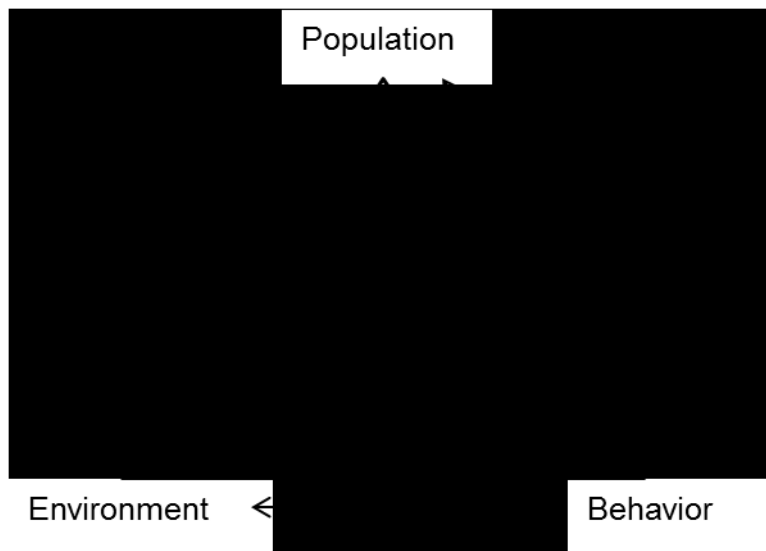


Figure 1. Triangle of Human Ecology. Adapted from (Meade 1977, 379-393).

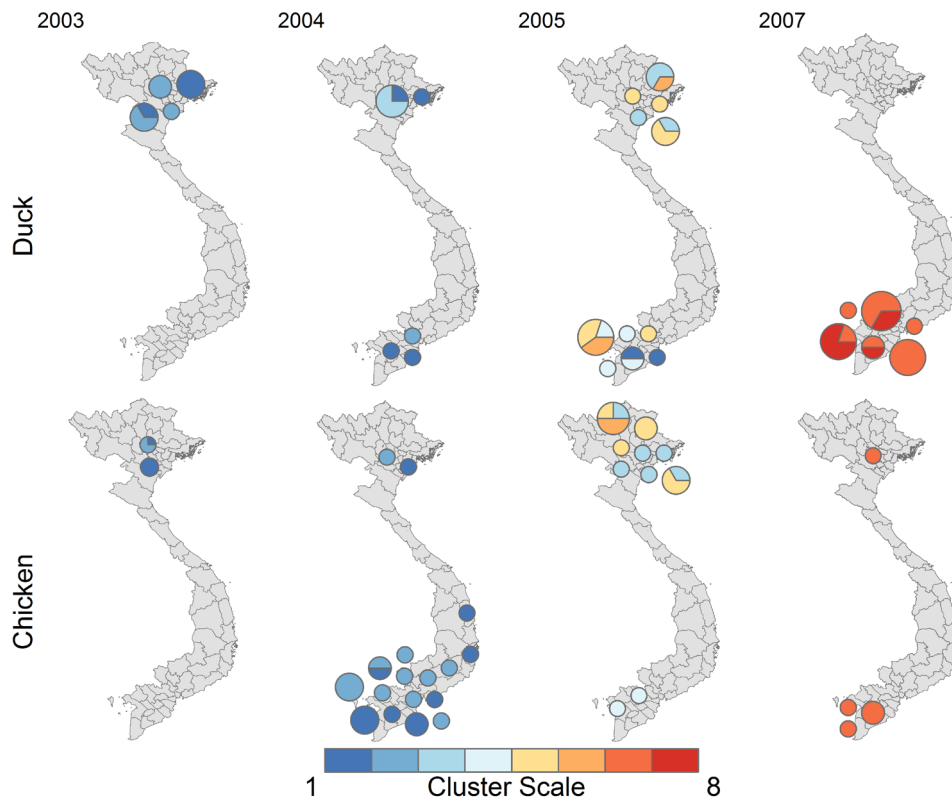


Figure 2. Duck and chicken viruses clustered according to genetic characteristics. Note cluster 8 which is comprised only of southern duck isolates. Cluster size is scaled to the number of viral isolates in each province. Note the absence of 2006, a year when no H5N1 was reported in Vietnam.

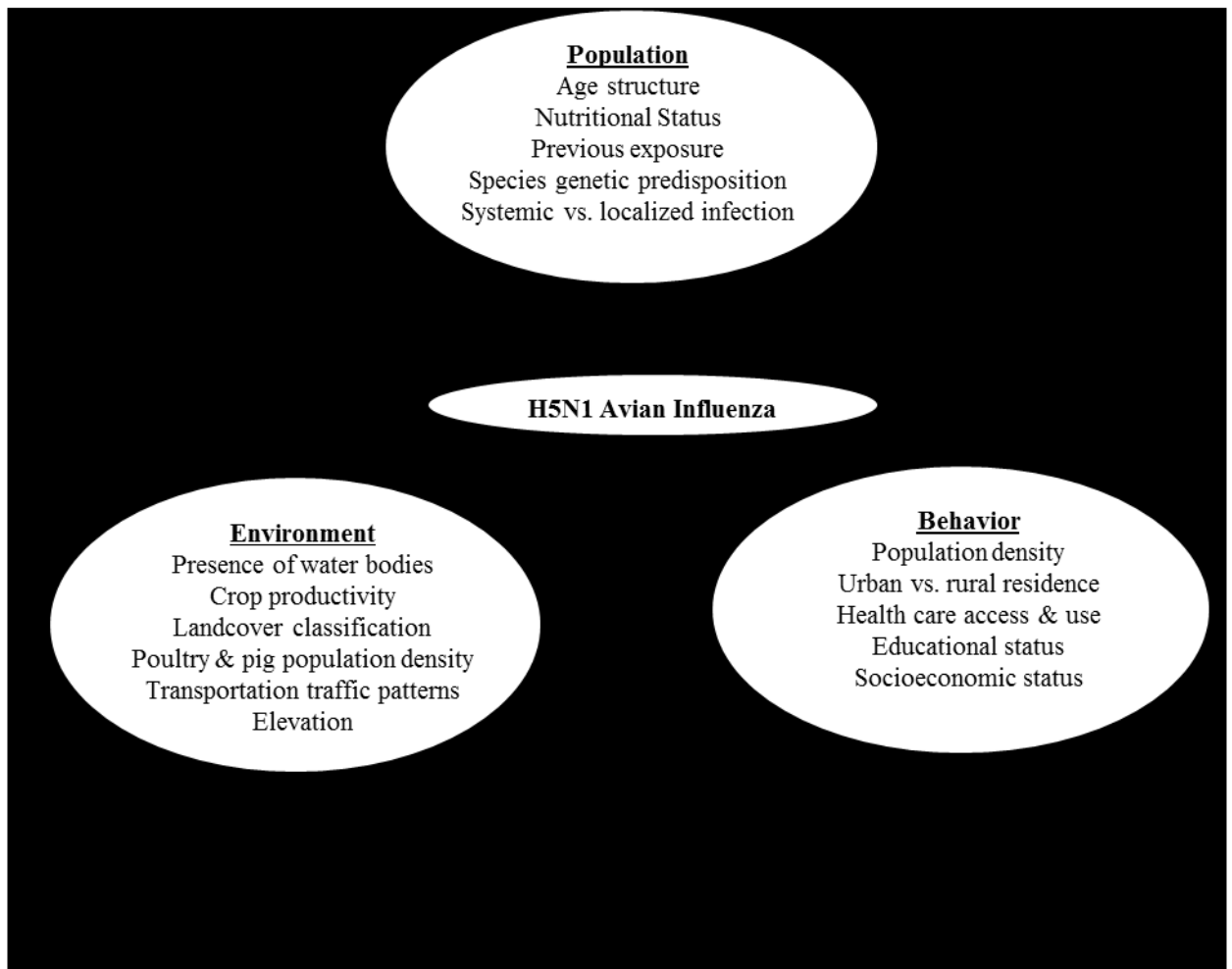


Figure 3. Triangle of human ecology as it looks for the ecology of H5N1 avian influenza in Vietnam.

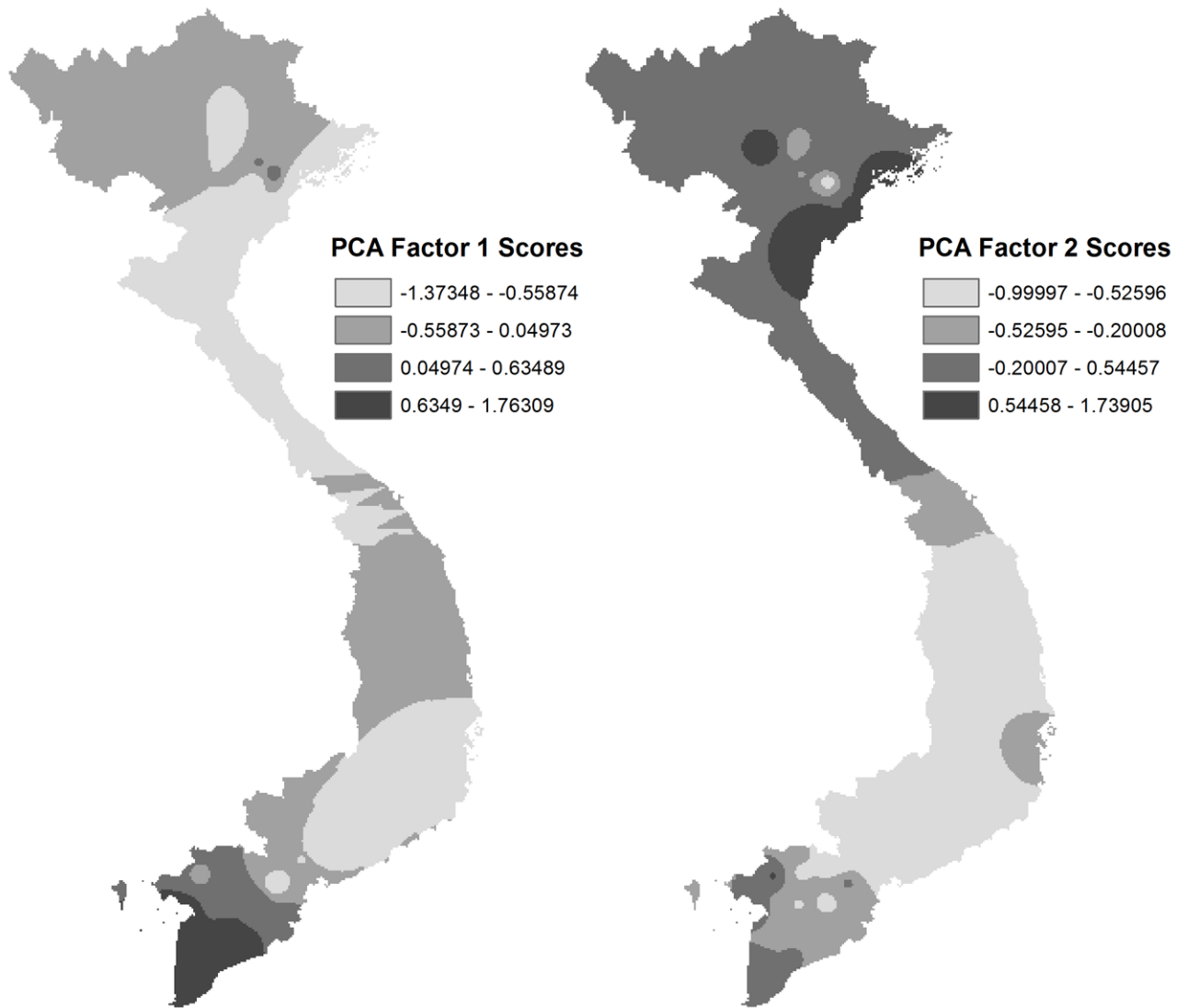


Figure 4. Interpolated PCA scores for the first two principal components, Factor 1 and Factor 2. Large changes in PCA scores for each factor over small geographic distances indicate potential spaces for barriers to gene flow.

Table 1

Examples of existing methodologies and available software for analysis of landscape genetic questions in medical geography.

Method	Purpose	Software
Analysis of Variance	Assess variation in genetic outcomes stratified by predictor variable.	R (<i>stats</i>)
Barrier Detection	Determine disruptions in gene flow, detect boundaries of genetic change. Multiple methods exist for determining barriers or boundaries to gene flow, including Wombling, Monmonier's Algorithm and Principal Component's Analysis (PCA).	Wombling: Passage, Wombsoft Monmonier's Algorithm: Barrier, R (<i>adegenet</i>)
Cluster Analysis	Explore clustering of genetic mutations in space and time. Link those clusters to underlying factors in the environment or population.	PCA: SPSS, R (<i>stats, psych</i>) SaTScan
Ecological Niche Modeling	Determine ecological niches where genetic mutations occur.	GARP, MaxENT
Geographically Weighted Regression	Explore the spatially varying influence of predictor variables on genetic outcomes.	GWR, ArcGIS 10
Mantel's Tests	Examine correlation between matrices, determining whether geographic and genetic distances between isolates are correlated. Geographic matrix can connect isolates along features such as rivers rather than across Euclidean space.	R (<i>ecodist, vegan</i>)
Moran's I	Assess the degree of spatial autocorrelation present in genetic outcomes at point or areal level.	GeoDa R (<i>ape</i>)
Multi-level Modeling	Explore the relative influence of variables at multiple scales (individual, community, landscape) on genetic outcomes.	SAS HLM
Non-Parametric Multi-Dimensional Scaling	Explore the relative influence of variables on multiple genetic indicators.	R (<i>ecodist, vegan</i>)
Spatial lag models	Relationship between genetic outcomes and predictor variables, controlling for effects of spatial autocorrelation.	GeoDa
Zero-Inflated Regression	Determine influence of predictor variables on count of mutations.	R (<i>pscl</i>)

Table 2

Average genetic distance for chicken versus duck H5N1 viral isolates according to gene segment and overall. For every gene segment the amount of genetic change in duck viruses is greater than for chicken viruses.

	PB2	PB1	PA	HA	NP	NA	MP	NS	Total
Chicken	0.06231	0.08903	0.07414	0.15683	0.07288	0.13373	0.07669	0.09722	0.76284
Duck	0.08734	0.1244	0.09004	0.18726	0.09993	0.18056	0.10223	0.15207	1.02384

Table 3

Summary of the MANOVA using eight genetic distance measures as the dependent variable and species, temporal distance and geographic distance as explanatory variables. Wilks λ is a measure of the amount of variance accounted for in the dependent variable by the independent variable; the smaller the value, the larger the difference between the groups being analyzed. The approximate F statistic is representative of the degree of difference in the dependent variable created by the independent variable, taking into account the covariance of the variables.

	Wilks λ	approx F	Pr(>F)
Species	0.345	22.52	< 2.2E-16***
Temporal Distance	0.078	139.432	< 2.2E-16***
Geographic Distance	0.477	13.037	1.59E-12***
Species:Temporal Distance	0.827	2.484	0.01715*
Species:Geographic Distance	0.909	1.186	0.31557
Temporal Distance: Geographic Distance	0.386	18.915	< 2.2E-16***
Species:Temporal Distance:Geographic Distance	0.934	0.841	0.56861

Significance values: 0

0.0001

**
0.01

*
0.05

Table 4

Environment and population variables considered in the analysis.

Variable	Time Period	Source
Environment		
Poultry	2003-2007	§§
Pigs	2003-2007	§§
Planted area of rice paddy	2003-2007	§§
Yield of rice paddy	2003-2007	§§
Water surface for aquaculture	2003-2007	§§
Water surface area	1981-1994 composite	**
Urban/built surface area	1981-1994 composite	**
Waterway freight traffic	2003-2007	§§
Roadway freight traffic	2003-2007	§§
Elevation	2000	‡‡
Population		
Population density	2005	††
High school graduates	2003-2007	§§
Rural population	2003-2007	§§
Urban population	2003-2007	§§
Medical professionals	2003-2007	§§
Passenger road traffic	2003-2007	§§
Average income	1999	§§

§§ General Statistics Office of Vietnam,

** GLCF (UMD)

‡‡ SRTM30 (NASA),

†† CIESIN

Table 5

Regression results showing the influence of six population-environment independent variables on viral NMDS loading scores across the three dimensions of the scaling (D1-D3).

	D1			D2			D3		
	Coefficient	t-Statistic	p-Value	Coefficient	t-Statistic	p-Value	Coefficient	t-Statistic	p-Value
Intercept	-8.93636	-4.98	<0.0001	-13.83283	-7.03	<0.0001	-0.15703	-0.08	0.9336
Temporal Distance	-1.28554	-7.52	<0.0001	4.43109	23.63	<0.0001	-1.14879	0.18	<0.0001
Environment									
Aquaculture	-0.00962	-3.04	0.0029	0.00357	1.03	0.3055	-0.00678	-2.05	0.0431
Percent Water	9.46783	2.26	0.0255	-7.1416	-1.56	0.1222	10.04685	2.29	0.0236
Population									
High School Graduation	0.13265	6.16	<0.0001	-0.00125	-0.05	0.9578	0.05299	2.35	0.0205
Population Density	-0.00051	-0.91	0.3667	0.00267	4.31	<0.0001	-0.00076	-1.29	0.1984
Low Income	0.01858	3.26	0.0015	0.00435	0.7	0.4884	-0.00454	-0.76	0.4488

R-squared for each model was: D1=0.70, D2=0.88, D3=0.49