

# LANDSCAPE GENETICS OF INFECTIOUS DISEASES: AVIAN AND SWINE INFLUENZA ETIOLOGY AND MALARIA VACCINE EVALUATION CASE STUDIES

**Michael Emch**  
**University of North Carolina at Chapel Hill**



# LANDSCAPE GENETICS

Interdisciplinary field premised on the idea that *exploring spatial variation in genetics* can illuminate *how organisms exist in and move across the landscape*.

Two steps to a landscape genetics analysis.

First: Pattern

How genetic characteristics vary in space,

Second: Process

Correlate those patterns with specific characteristics of the underlying landscape.

Goal: Identify the landscape factors that drive evolutionary processes.

Which in turn enables scientists to predict future developments of genetic diversity.

## SO FAR...

Primarily focused on plants and animals rather than pathogens or people.

Infer colonization patterns, habitat restriction and extinction events of mammals, reptiles, insects and plants.

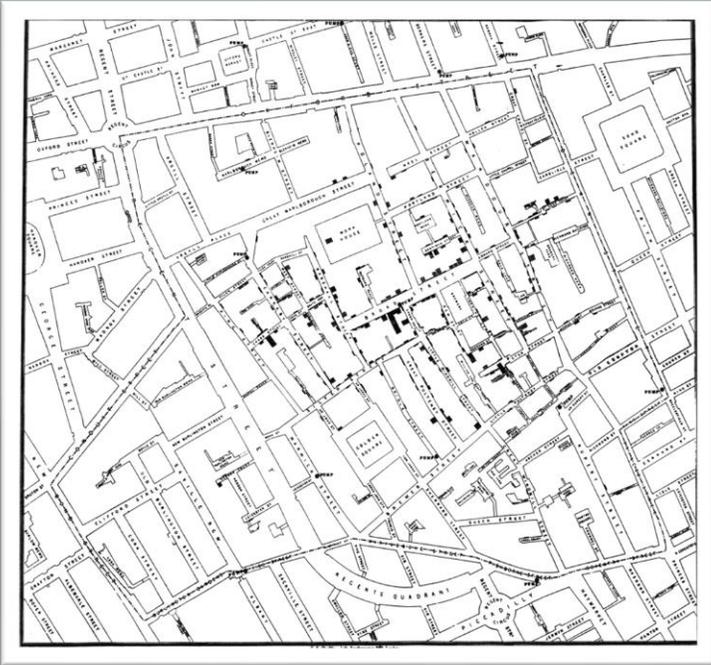
More recent recognition that landscape genetic techniques can be used to explore drivers of disease spread and parasite transmission and gene flow as they relate to human illness.

# LANDSCAPE GENETICS OF INFECTIOUS DISEASES

Answer questions about what features of human-natural landscapes drive disease emergence and pathogenic evolution.

What local level population and environment variables are related to molecular evolution of pathogens? Or act as barriers to spread of pathogens? Or drive diffusion of new variants? What is the impact of vaccination campaigns?

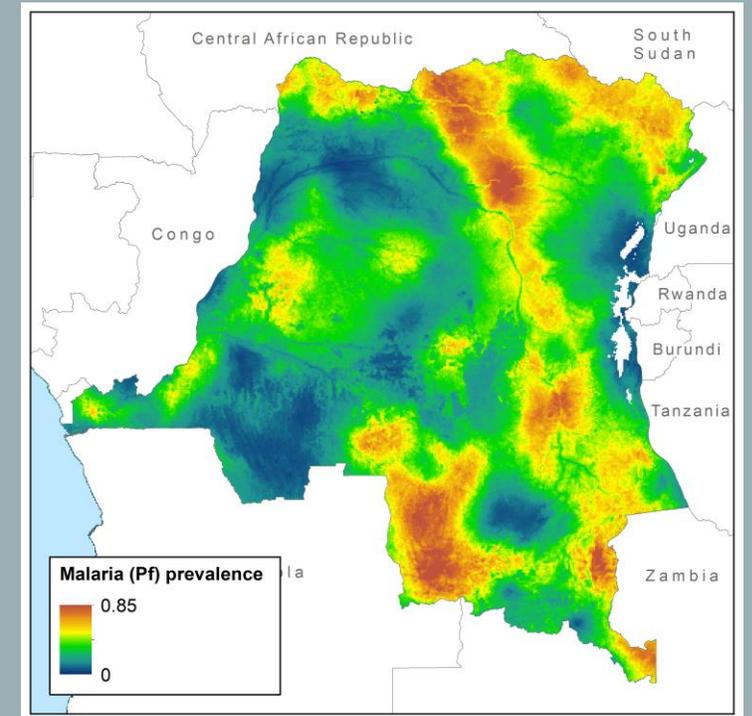
# JOHN SNOW 2.0: GEOGRAPHY OF PATHOGENS: SPATIAL AND MOLECULAR EPIDEMIOLOGY



**John Snow's 1854 Cholera Map**

Development of **GIS** and **PCR** has enabled integration & analysis of spatially referenced genetic descriptions of pathogens

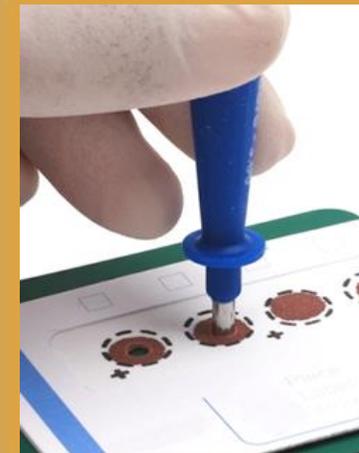
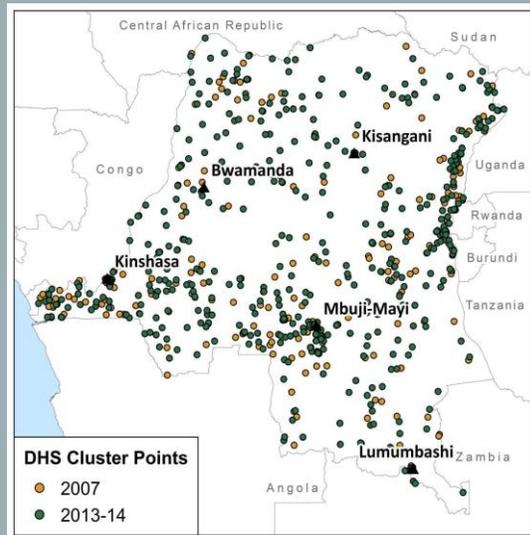
Big Data: both spatial and genetic



***P. falciparum* Malaria in 2013  
Democratic Republic of Congo (DRC)**

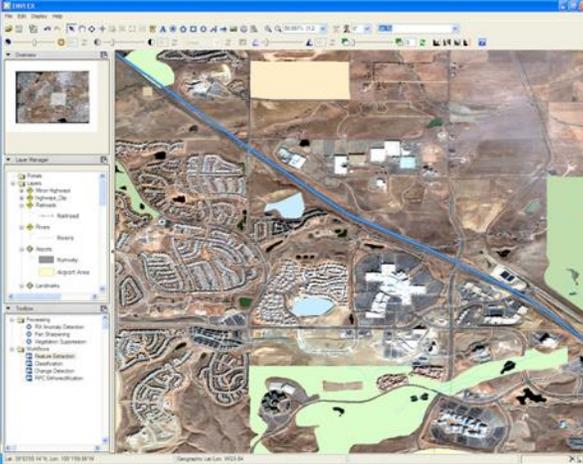
# DARWIN 2.0: COMBINING SPATIAL AND GENETIC INFORMATION USING BIOMARKERS

Finer spatial and temporal scales made possible by high-resolution molecular and geospatial datasets.



DRC Demographic and Health Survey Blood Spots

# TECHNOLOGY: MEASURING SPACE AND PLACE



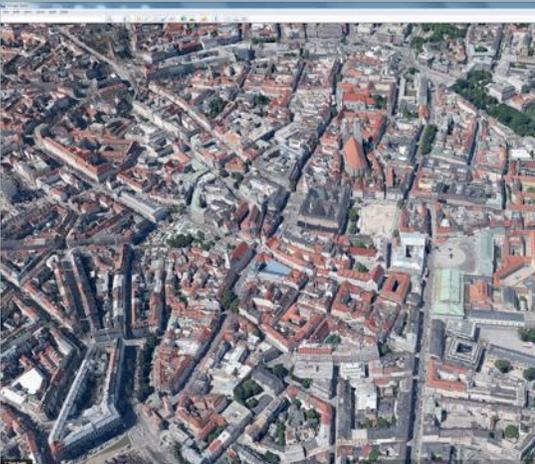
Geographic Information Systems (GIS)



Global Positioning System (GPS)



GPS Tracker



Google Earth



GPS-enabled Tablets and Phones



Satellite Imagery

# Don't Reinvent the Wheel: Studies of Space, Place and Health and Studies Not On Health

## NAS Members



Michael Goodchild



Waldo Tobler



Sir Peter Haggett

## Health Geographers



SV Subramanian

Harvard



Sara McLafferty

U Illinois



Dan Brown

U Washington

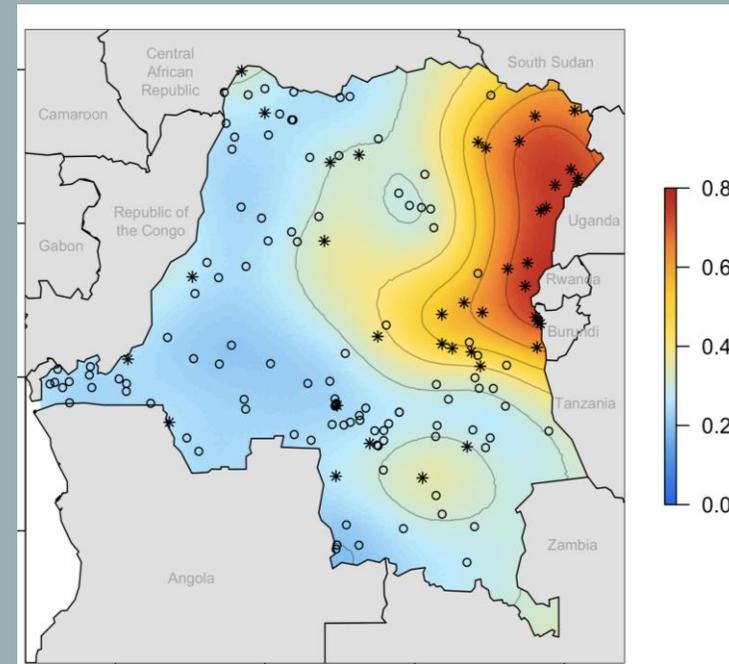
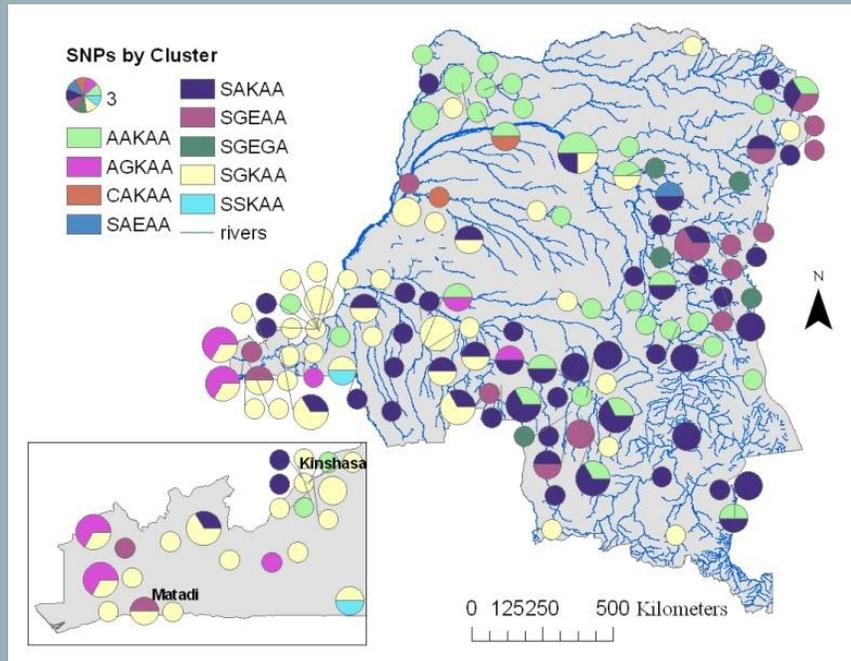


Andrew Cliff

Cambridge

# Landscape Genetics of Malaria Drug Resistance in the DRC

Maps show that drug resistance mutations are heterogeneous but have regional patterns for Sulfadoxine/Pyrimethamine (SP)

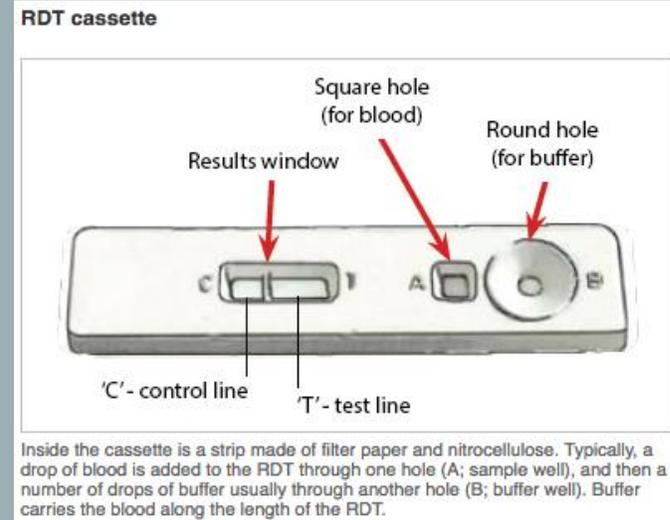
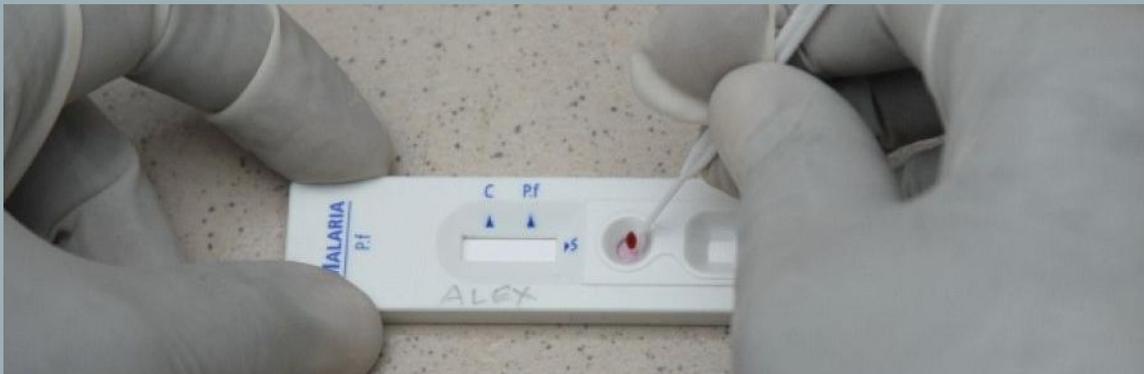


Geographic distribution of *dhps* haplotypes. Some of the *dhps* gene mutations are associated with SP resistance.

SP resistant malaria

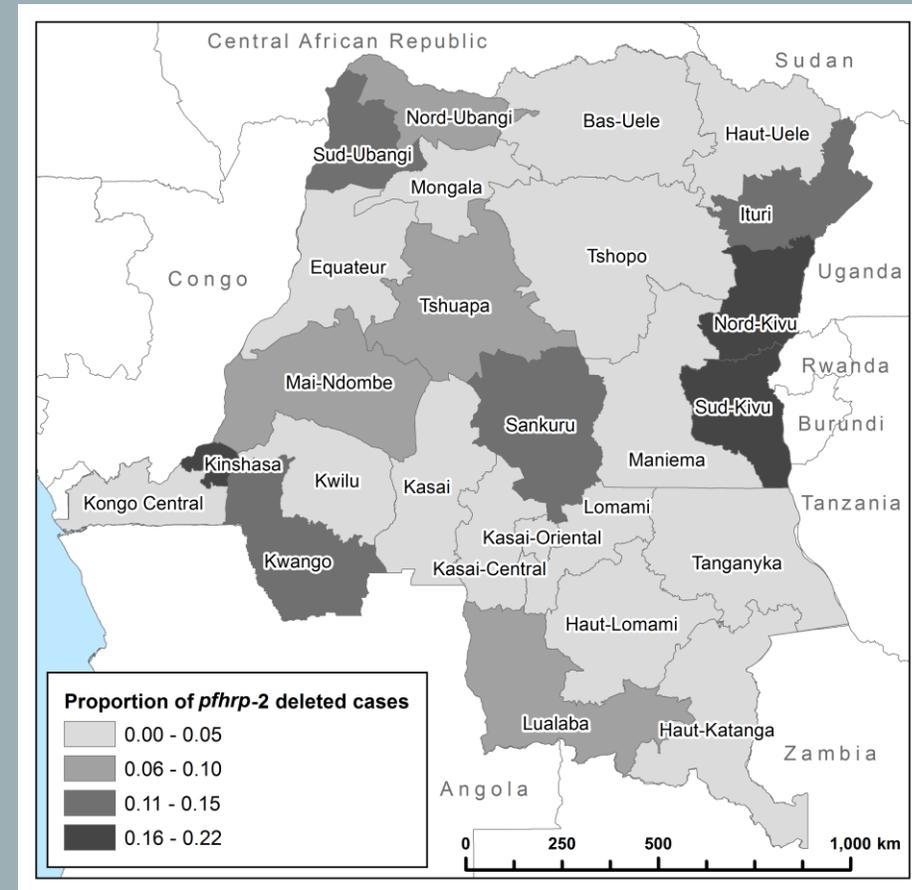
# STEALTH MALARIA

- Rapid diagnostic tests (RDTs) account for more than two-thirds of malaria diagnoses in Africa.
- Deletions of the *Plasmodium falciparum* hrp2 (pfhrp2) gene cause false-negative RDT results.
- Spread of hrp2-deleted *P. falciparum* mutants would represent a serious threat to malaria elimination efforts.

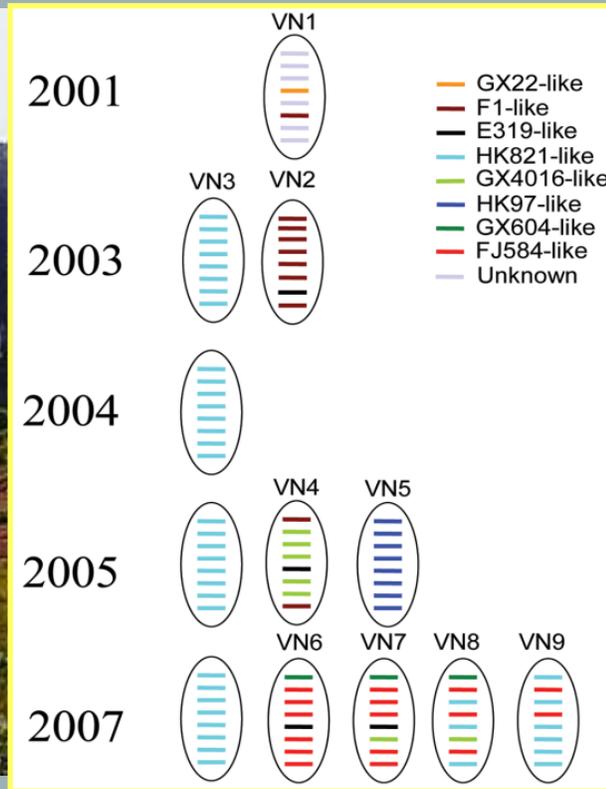


# Distribution of hrp2-deleted *P. falciparum* parasites

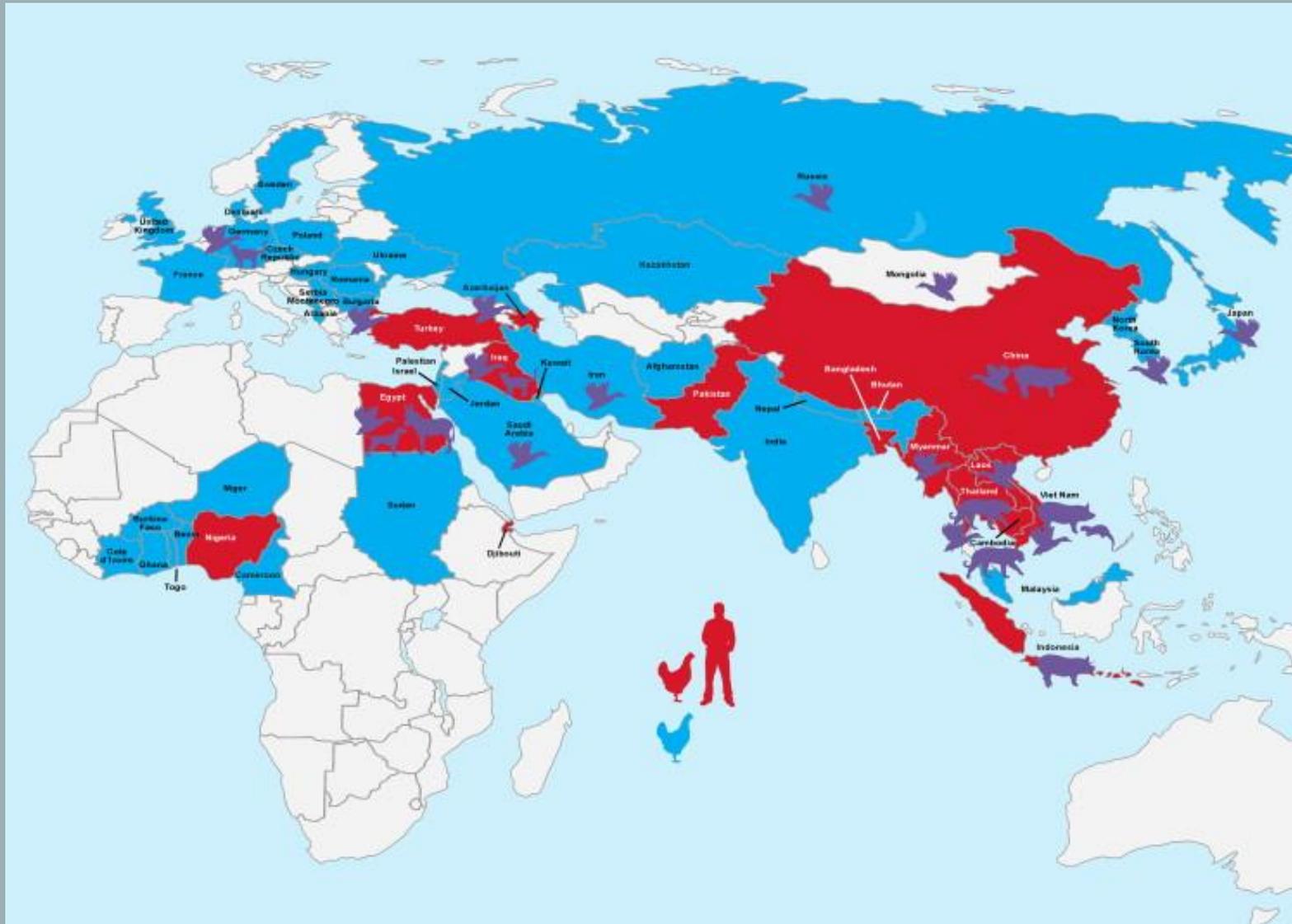
- We identified 149 hrp2-deleted parasites, representing 6.4% of all *P. falciparum* infections in the DRC.
- Spatial approach to understand the distribution and ecological drivers of the deletions.
- Significant clustering of pfhrp2 deletions near Kinshasa and Kivu.
- More common in low malaria transmission areas.



# LANDSCAPE GENETICS IN AVIAN INFLUENZA ETIOLOGY RESEARCH



# Areas reporting H5N1 Avian Influenza Cases

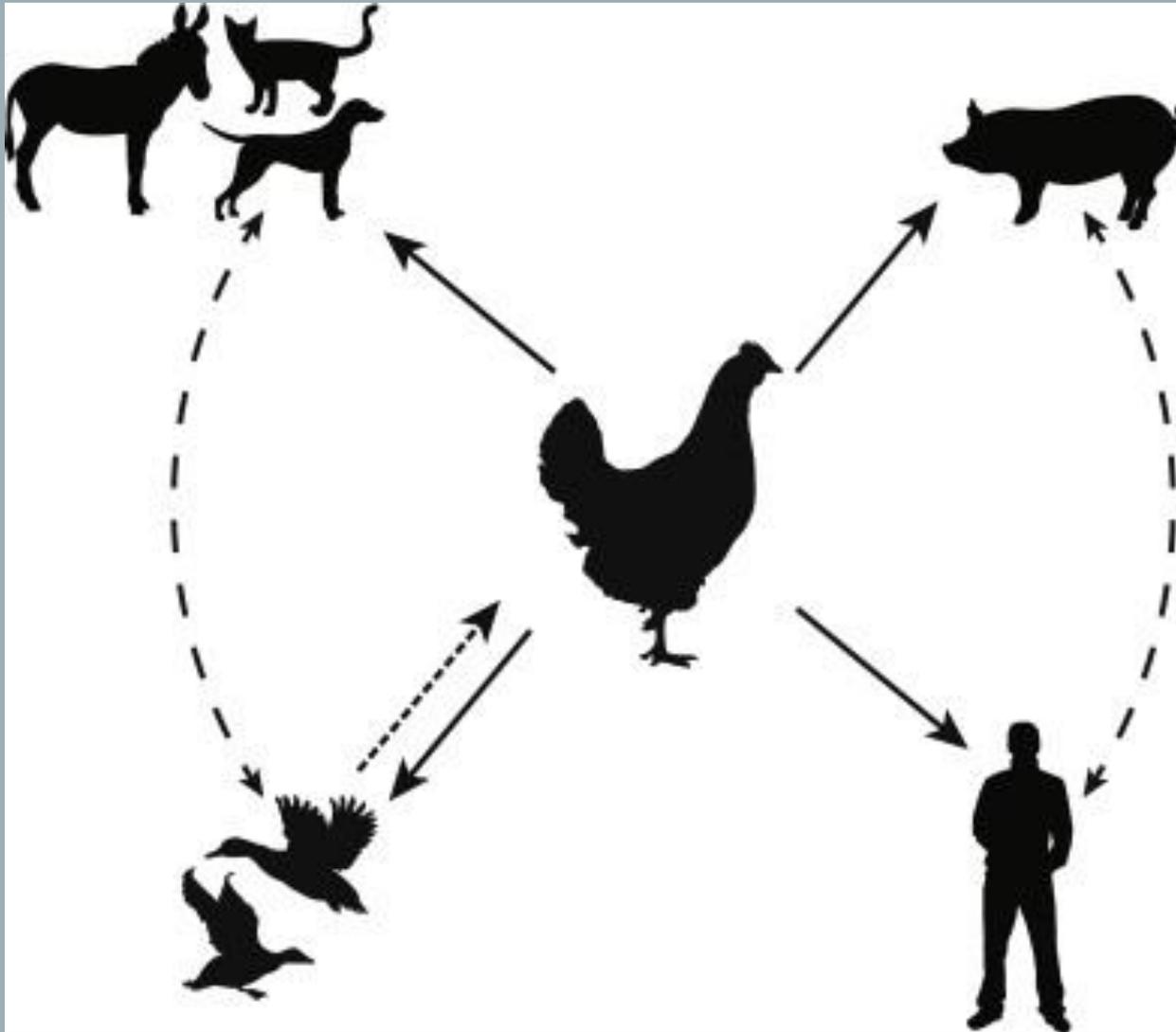


Countries shaded in red have concurrent H5N1 outbreak in domestic poultry and humans.

Blue countries signify outbreaks only in wild avian species.

Additional cases of mammalian H5N1 are depicted as shaded figures.

# Routes of H5N1 transmission



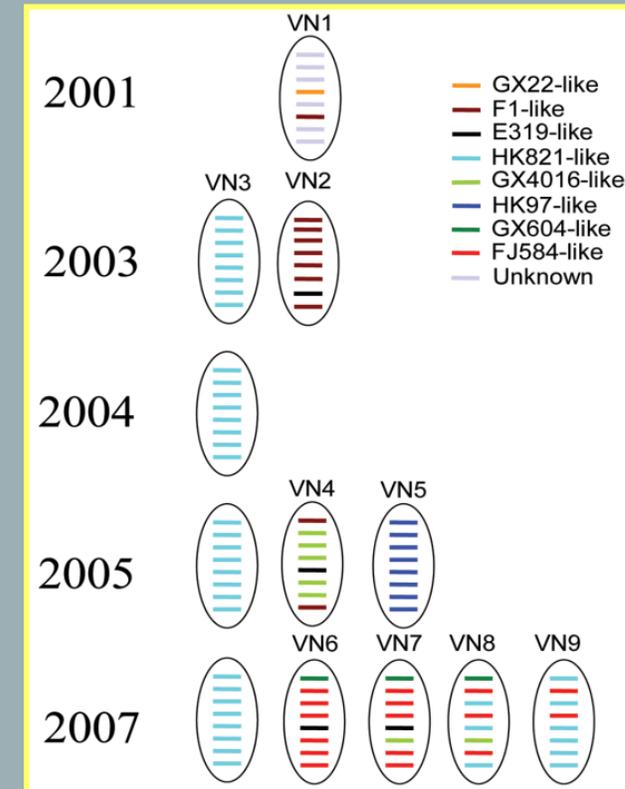
H5N1 influenza viruses cross the species barrier through close contact with infected birds, in particular domestic poultry.

Contact with domestic poultry (chickens, ducks, etc.) has resulted in the inter-species transmission of H5N1 to non-avian hosts.

Some transmission of H5N1 from wild migratory birds to domestic and/or captive mammals.

# H5N1 IN VIETNAM

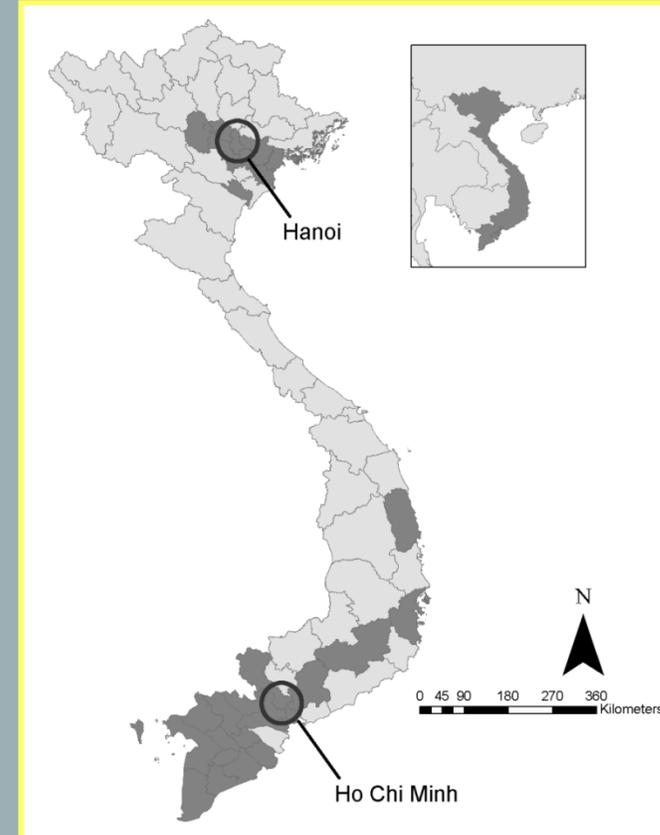
- Major outbreak in 2003
  - Source of introduction unknown, legal or illegal poultry trade from China is suspected source
- Factors influencing H5N1 in Vietnam:
  - Shared border with China
  - Cultural preference for live or freshly killed poultry
  - Ecosystem: poultry integral to agricultural production in rural Vietnam, over 80% of rural Vietnamese have backyard poultry flocks



HPAI H5N1 genotypes from introduction and reassortment in Vietnam from 2001 to 2007 (Wan et al., 2008).

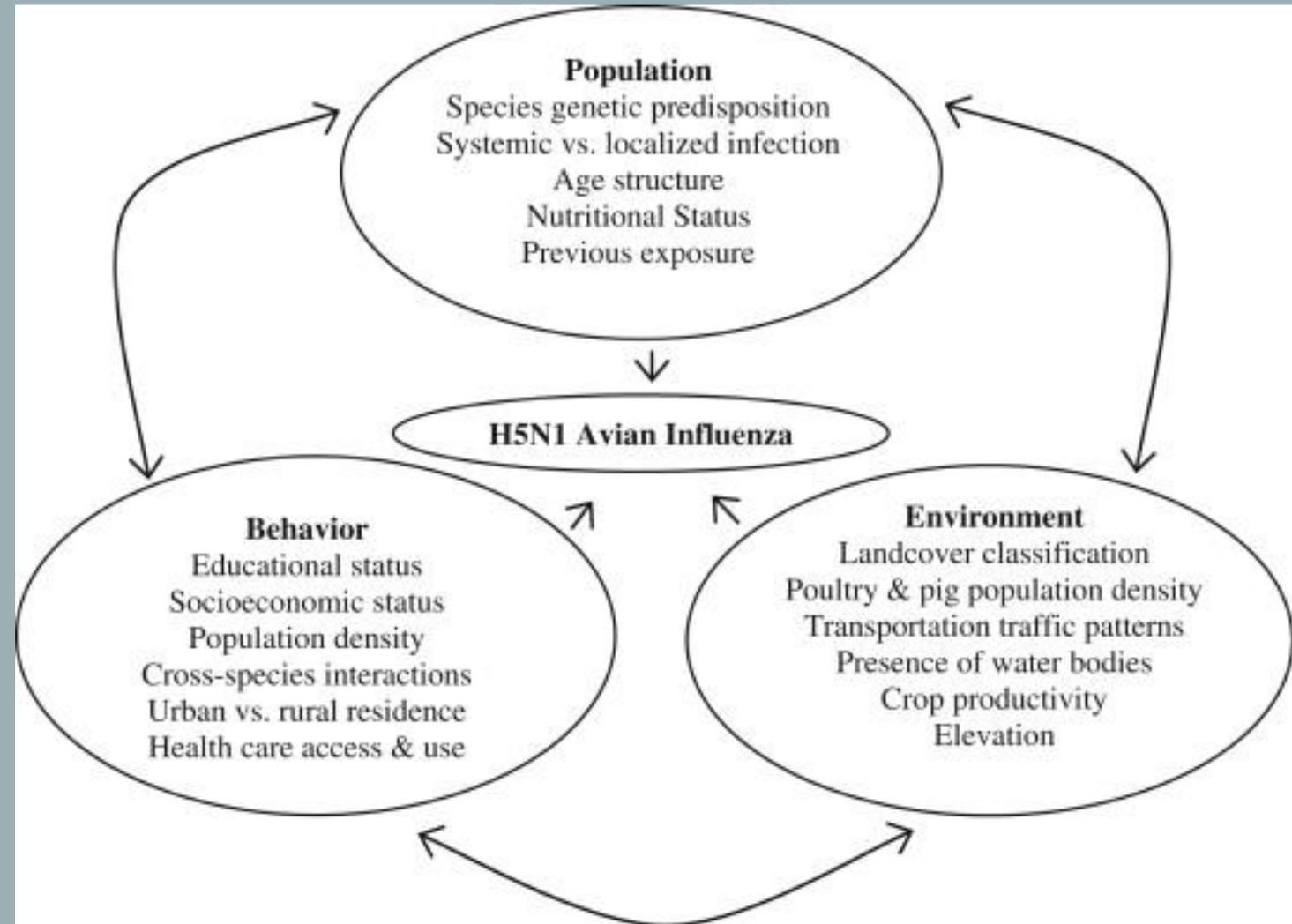
# H5N1 AVIAN INFLUENZA DATASET

- 110 cases of H5N1 from Vietnam
  - From the National Center for Veterinary Diagnosis (NCVD), Hanoi and from GenBank
- For each case there is:
  - Fully sequenced genetic data
    - All eight gene segments descendant from the A/duck/Hong Kong/821/2002 virus, a stable lineage likely resulting from single introduction (Dung Nguyen et al., 2008; Smith et al. 2006)
  - Province-level location of incidence
  - Year of incidence (2003-2007)
    - Note: no cases in 2006, a year of heavy vaccination campaigns in Vietnam
  - Species of isolation (53 chicken, 57 duck)



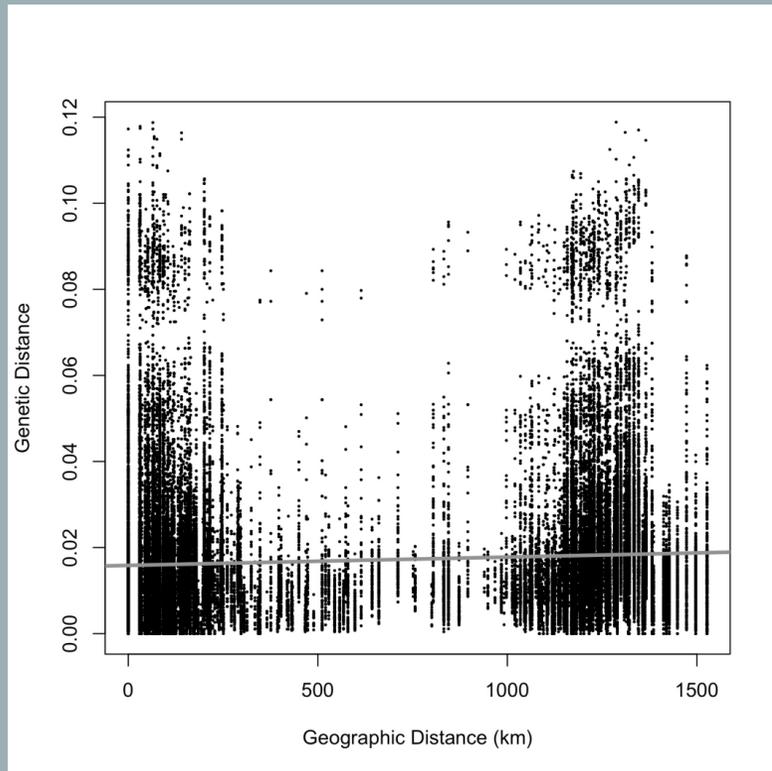
Darkened provinces indicate locations of virus isolation.

- We studied what elements in the social and natural environments of the Vietnamese landscape were driving H5N1 genetic variation.
- H5N1 avian influenza is an anthroozoonotic pathogen and that the majority of infected birds in Vietnam are living as domesticated animals in environments highly mediated by their human owners.
- The goal is to understand molecular change in H5N1 avian influenza viruses as the outcome of interacting environmental and social pressures of hypothesized drivers of molecular change.

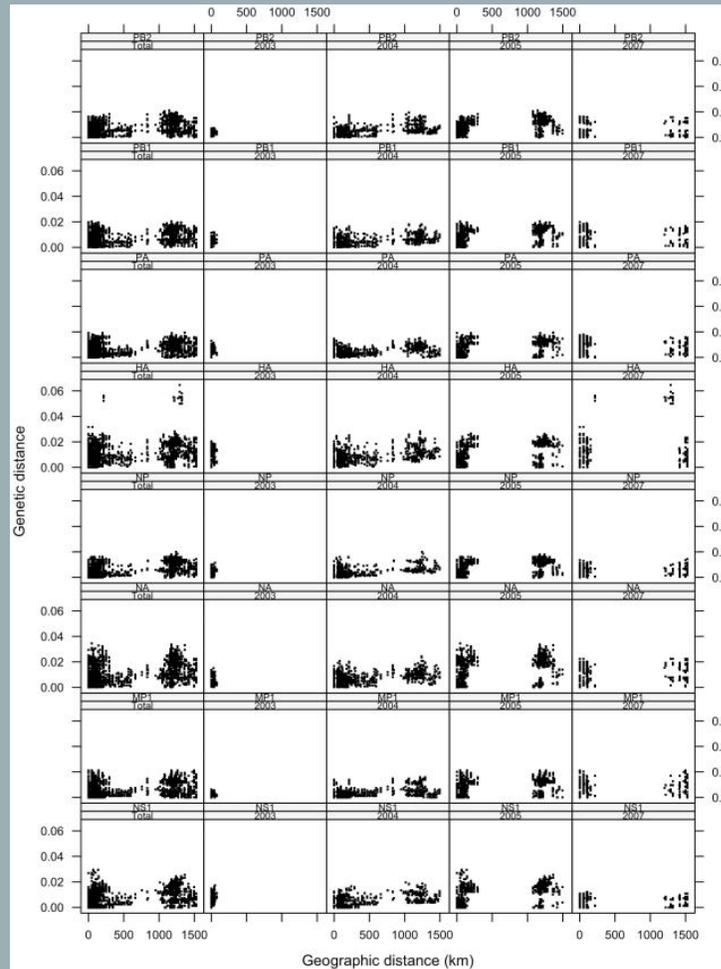


Framework describing the disease ecology of H5N1 avian influenza in Vietnam's domestic poultry.

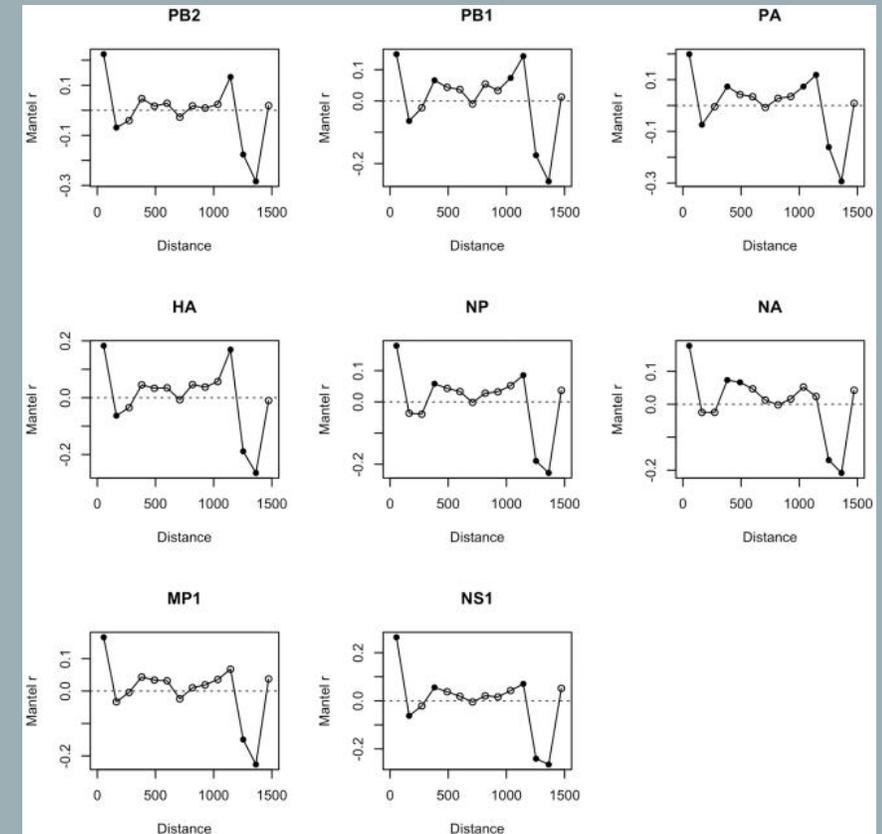
# GENETIC VS. GEOGRAPHIC DISTANCE: H5N1 IN VIETNAM



All cases, least squares line in grey



Stratified by year and protein



Mantel Correlograms stratified by gene segment

# SUMMARY

- Evolution of H5N1 viruses in Vietnamese domestic poultry is highly correlated with the location and spread of those viruses in geographic space.
- This correlation varies by time, and gene, though a classic isolation by distance pattern is observed.
- Variables relating to both the environmental and social ecology of humans and birds in Vietnam interact to affect the genetic character of viruses.
- A combination of suitable environments for species mixing, the presence of high numbers of potential hosts, and in particular the temporal characteristics of viral occurrence, drive genetic change among H5N1 AIV in Vietnam.

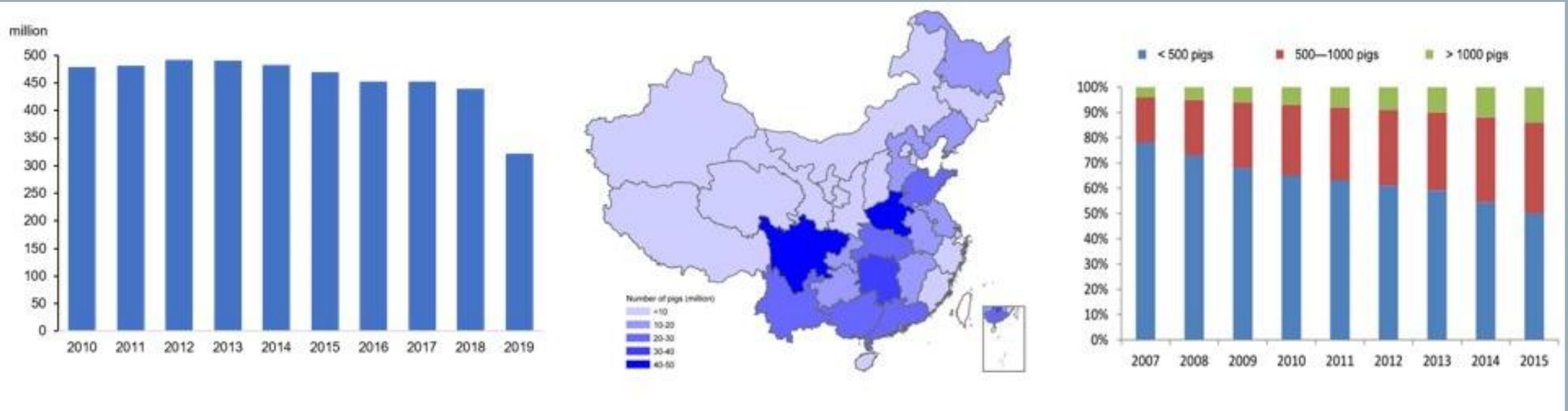
Wan et al. 2007 *Avian Diseases*. 51; Wan et al. 2007 *Bioinformatics*. 23(18); Wan et al. 2008 *PLoS One*. 3(10); Carrel et al. 2011 *Avian Diseases*. 6(4); Carrel et al. 2012 *EcoHealth*. 9(1); Carrel et al. 2012 *Health & Place*. 18(5); Carrel et al. 2010 *PLoS One*. (5)1.

# SWINE FLU IN CHINA AND UNITED STATES

**The central hypotheses of this study** are that:

- 1) novel swine influenza viruses (SIVs) (e.g., avian-like novel H1N1 and H3N2v viruses) continue to emerge and evolve to be highly transmissible viruses,
- 2) intercontinental movement of genetic segments in the US and China will further diversify the swine influenza A virus (IAV) population and facilitate emergence of novel SIVs, and
- 3) unique ecological factors drive evolutionary dynamics of IAVs in the two largest swine industry systems.

# SWINE INDUSTRY IN CHINA

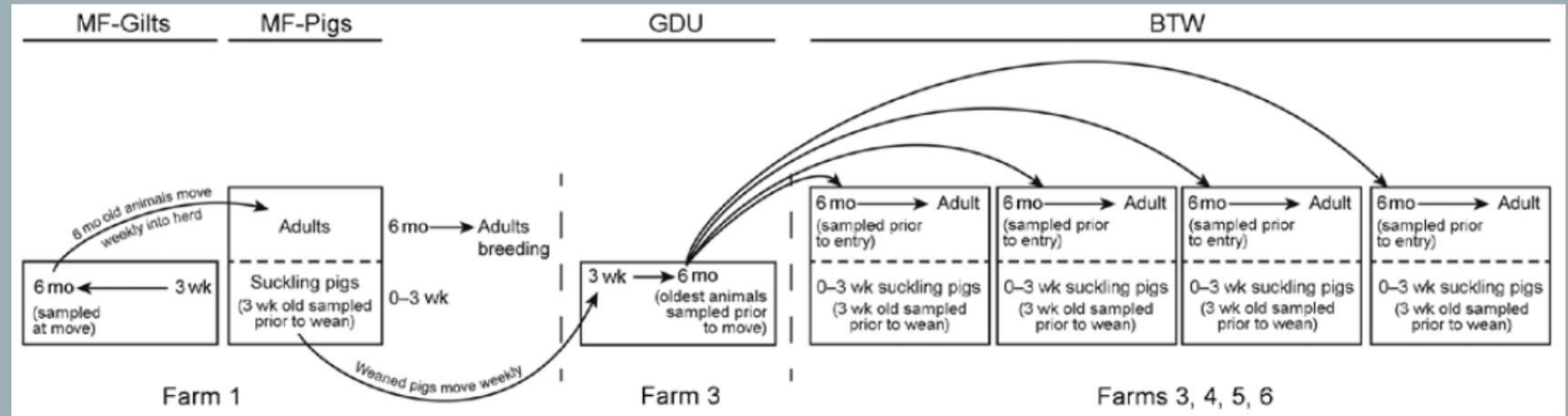


# of pigs produced

Distribution of farms

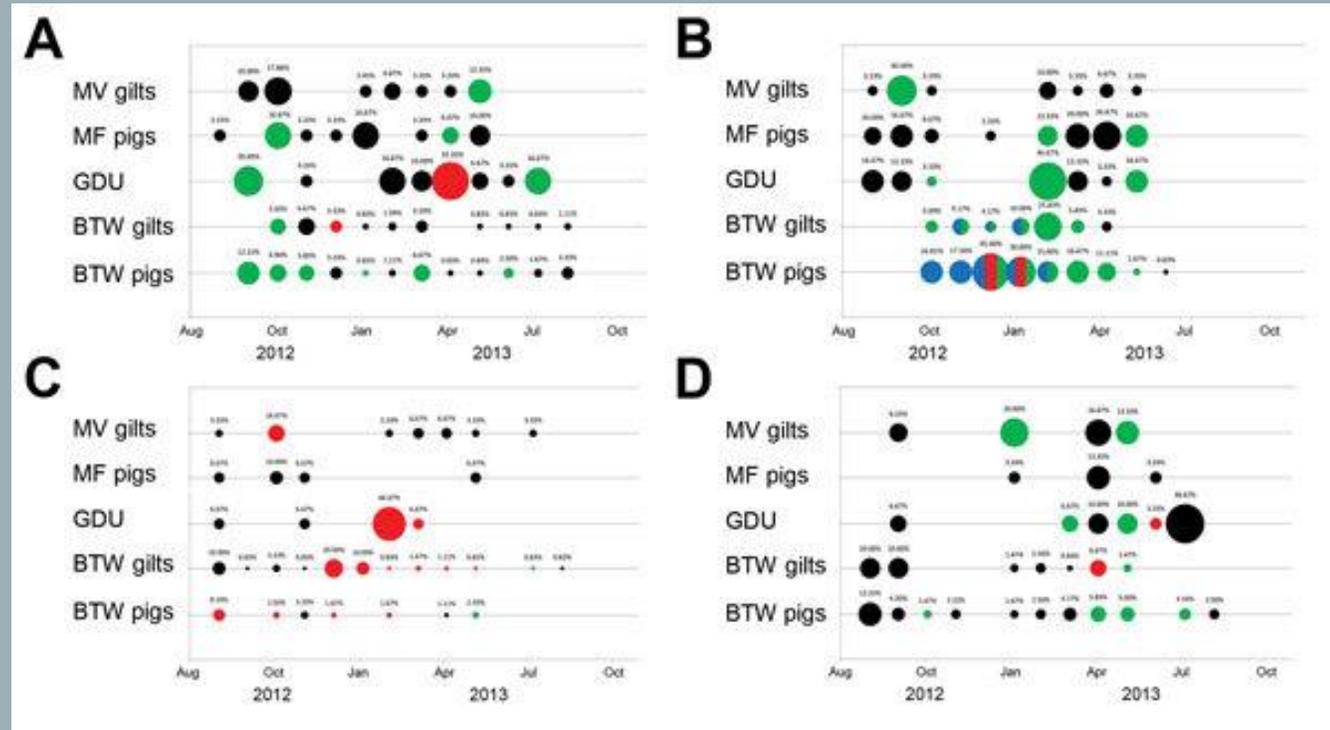
Farm sizes

# U.S.: Prevalence of IAV varies by system and farm type.

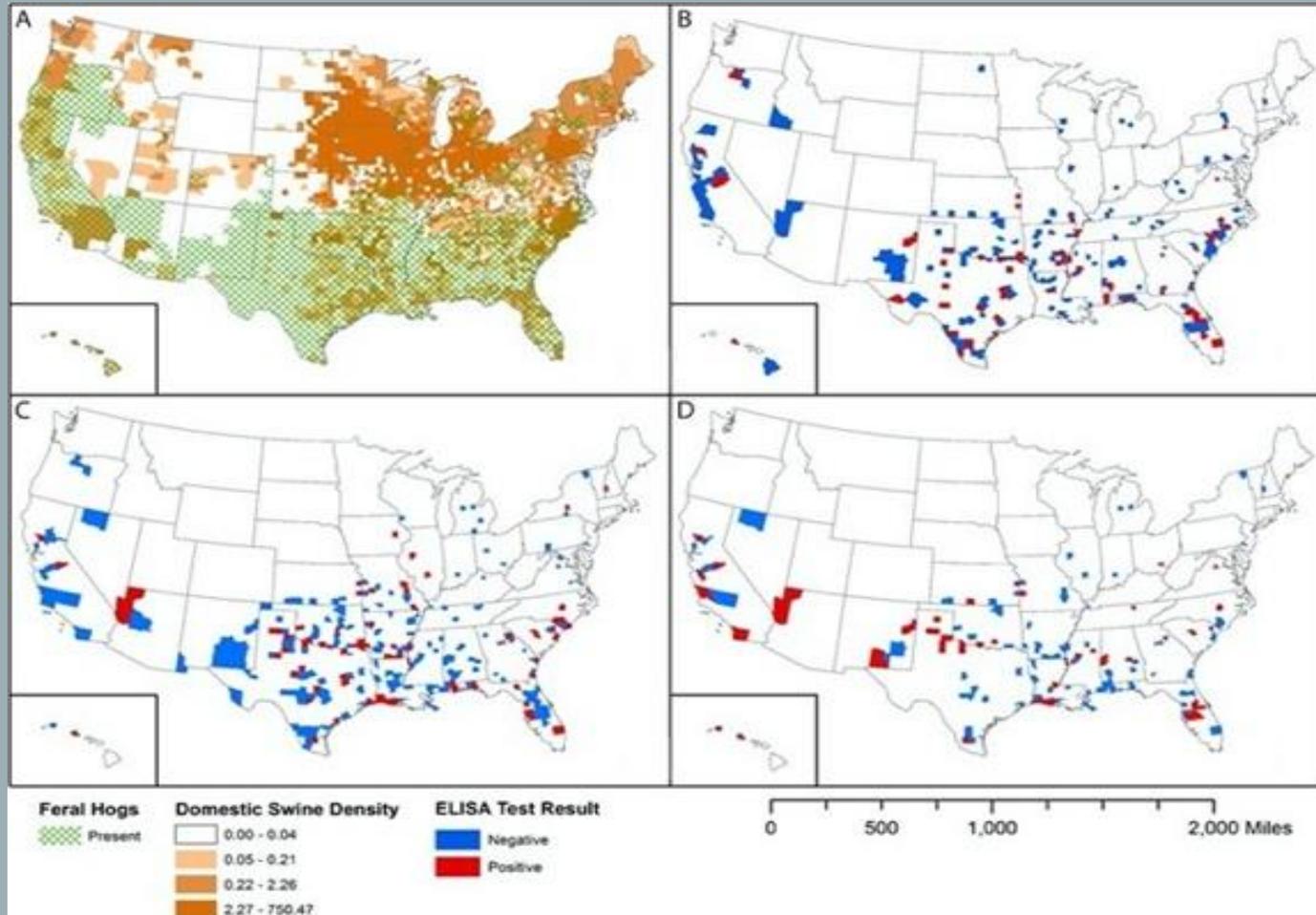


Month of positive influenza samples in Illinois, Georgia, Oklahoma, Nebraska.

Influenza A subtypes indicated by circle color: green, H1N1; blue, H1N2; red, H3N2; black, untyped. Multicolored circles indicate the detection of >1 subtype.



# DOMESTIC/ FERAL SWINE IN THE U.S.



**A: Geographic distribution of domestic and feral swine in the U.S.**

**B to D: Distributions of IAV ELISA-negative and -positive feral swine serum samples- 2011, 2012, 2013.**

# Emerging of avian-like H1N1 swine influenza viruses in China (2011-2018)

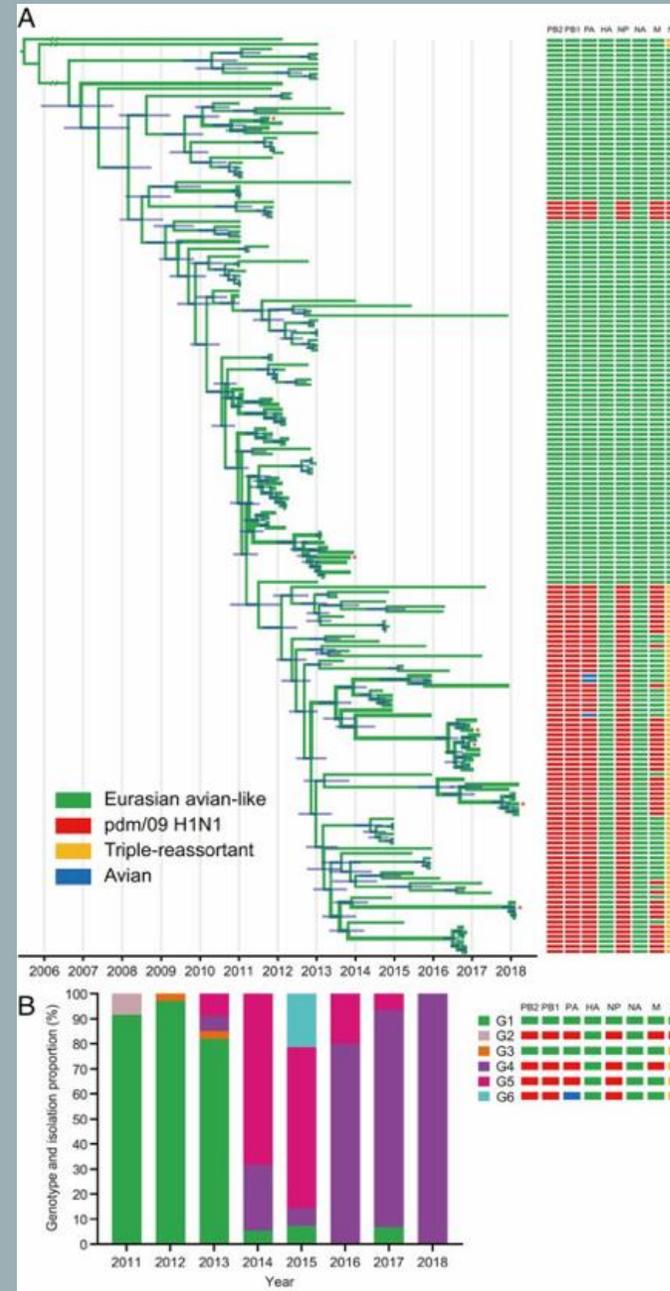
Recently emerged genotype- reassortant of avian-like H1N1 virus.

Increased human infectivity: 10.4% of swine workers were positive for virus acquired.

Greatly increases the opportunity for virus adaptation in humans and raises concerns for the possible generation of pandemic viruses.

Sun et al. 2021, *PNAS*.

By China project collaborator, Prof. Jinhua Liu



A) Phylogenetic analysis of Eurasian H1N1 SIVs in China from 2011 to 2018;

B) Diversity of genotypes of EA viruses isolated from swine in China, 2011–2018.

# DEEP LEARNING METHODS: SWINE FLU

- Measure the probability for a novel SIV to emerge and become enzootic.
- Emergence risk quantifies the probability for a novel enzootic IAV viruses (genetic pool) and an ecological setting.
- Application of deep learning to extrapolate virus emergence and trajectory dynamics of a novel SIV.

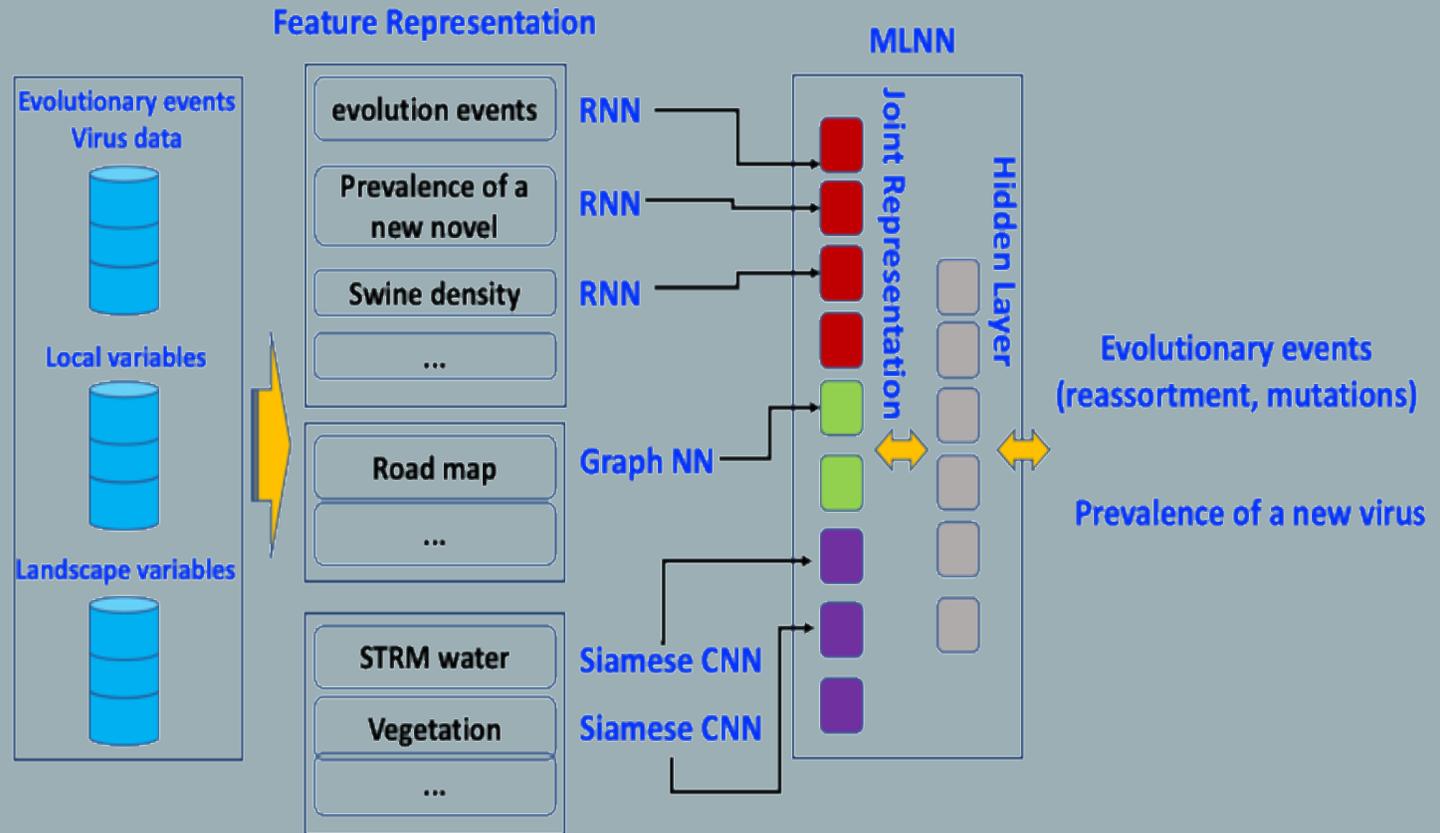
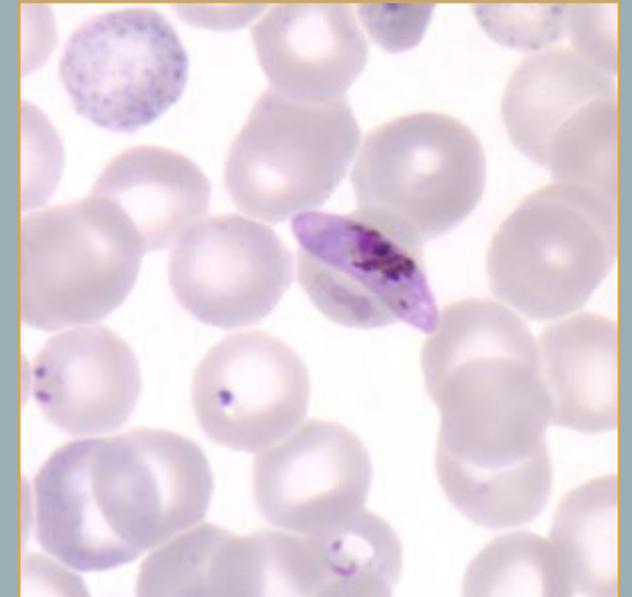
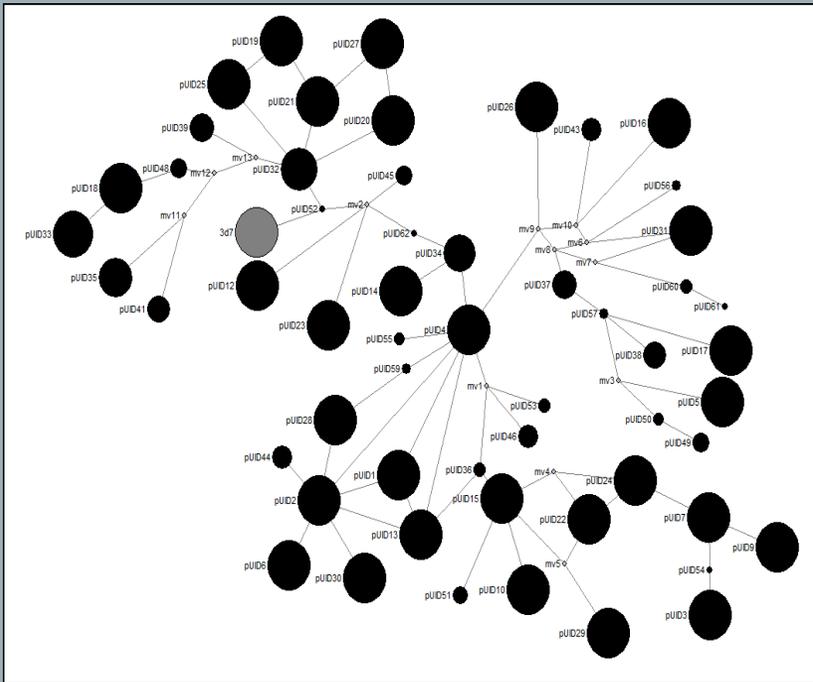


Fig. 9. MDL to predict emergence and trajectory dynamics of a novel SIV.

# LANDSCAPE GENETICS IN MALARIA VACCINE EVALUATION RESEARCH



Gametocyte of *P. falciparum*

# RTS,S MALARIA VACCINE

First vaccine for Malaria approved for use

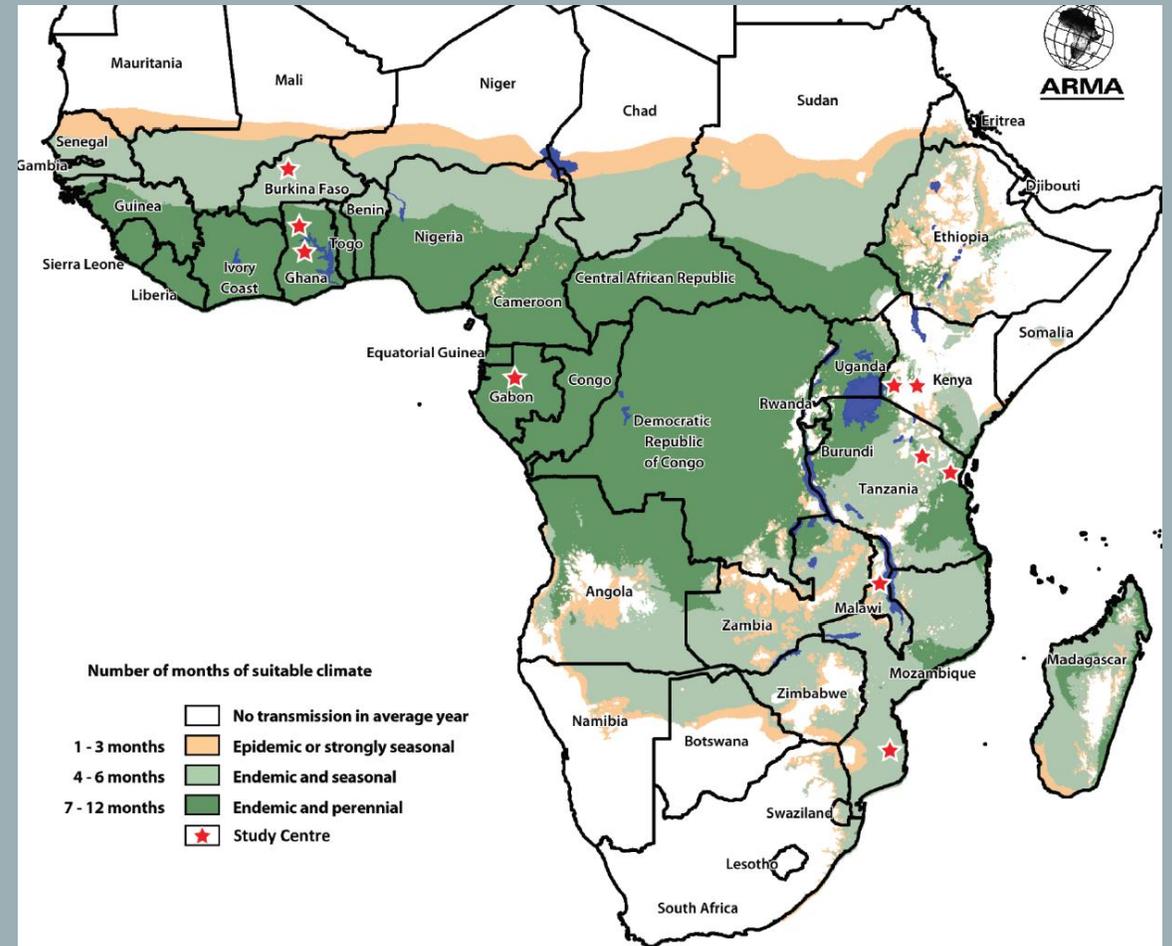
Phase III trials: 11 sites, 7 countries

Present study includes 3 sites:

Lilongwe, Malawi

Kintampo, Ghana

Lambarene, Gabon



## OVERALL PHASE 3 TRIAL EFFICACY

Efficacy against all episodes of clinical malaria was estimated  
36.3% effective against clinical malaria in children (5-17 months)

22% in Manhica, Mozambique

50.8% in Lilongwe, Malawi

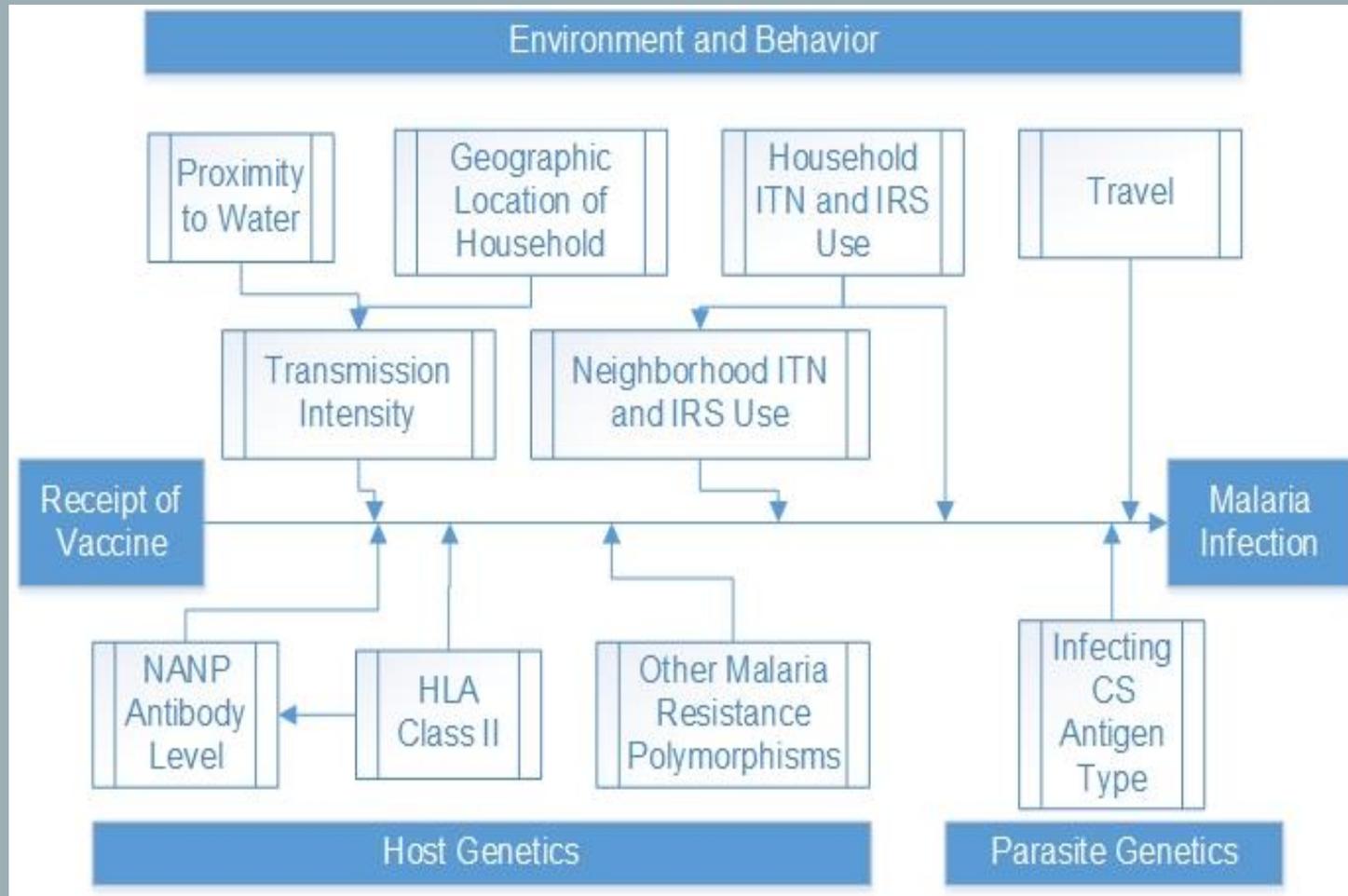
74.6% in Kilifi, Kenya

May be more effective in higher transmission intensity areas

Less effective in infants (6-12 weeks)



## CONCEPTUAL FRAMEWORK OF EFFECT MODIFIERS ON MALARIA VACCINE EFFICACY



Due to the modest efficacy of RTS,S/AS01e, the combinations of factors (ecological, parasite, human host) impacting its effectiveness must be clearly understood, as this information will be critical for implementation policy and future vaccine designs.

# ECOLOGICAL VARIABLES

**Table 4: Ecological variables.**

**Age**

**Sex**

**ITN Use by each family member**

**Malaria in household member**

**Household insecticide spraying**

**Proximity of water to house**

**Vegetation and type near house**

**Household wall materials**

**Malaria transmission intensity**

**Population density near house**

**Rainfall**

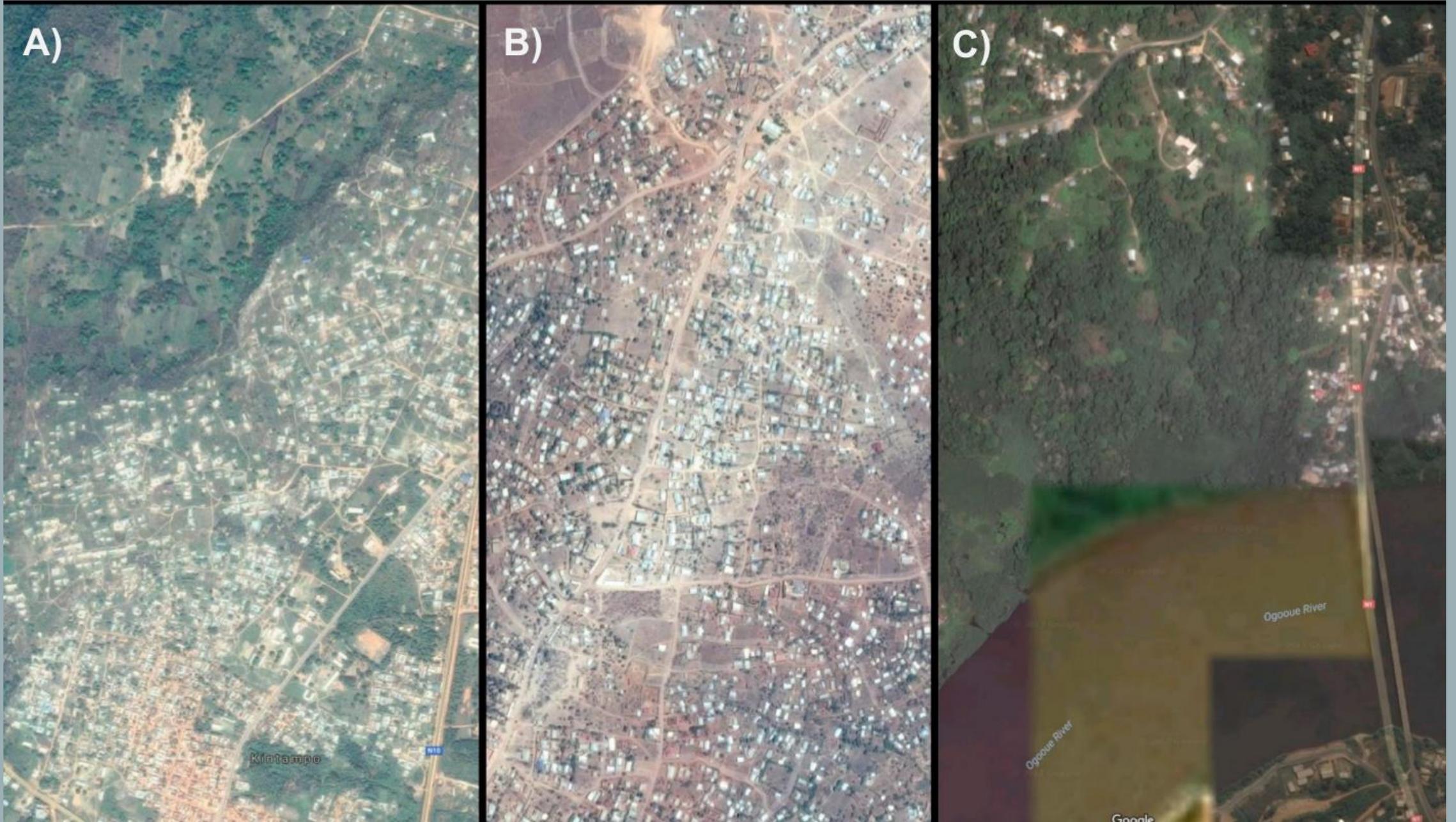
**Elevation**

**Land use land cover**

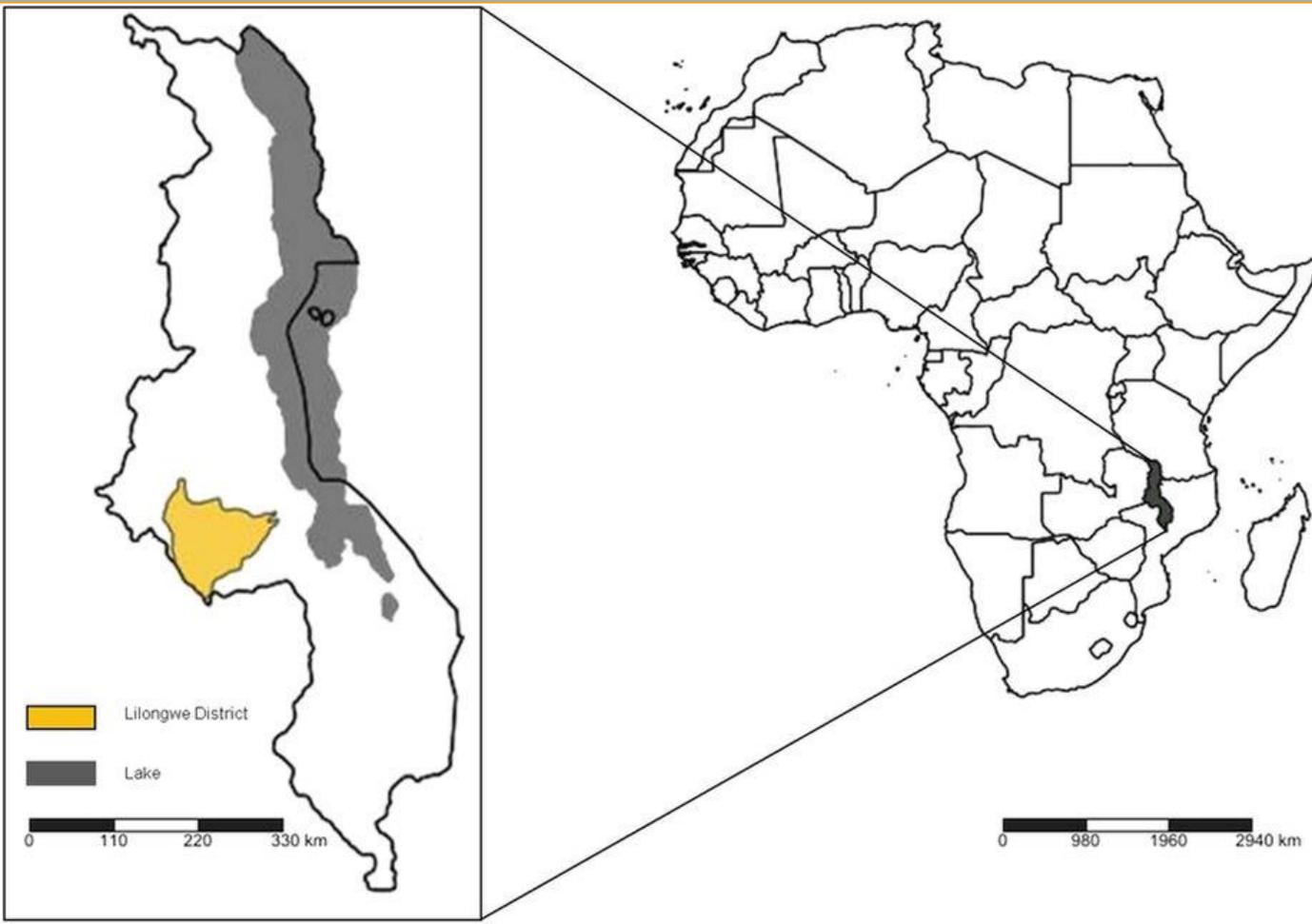
**NDVI and other vegetation indices**



**Figure 2: Google Earth images of sections of trial catchment areas in (A) Kintampo, Ghana, (B) Lilongwe, Malawi, and (C) Lambarene, Gabon.**

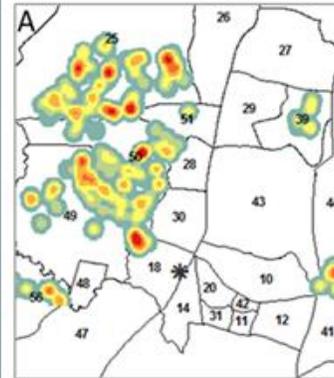


# WITHIN-SITE VARIATION OF BED NET USE AND MALARIA PREVALENCE



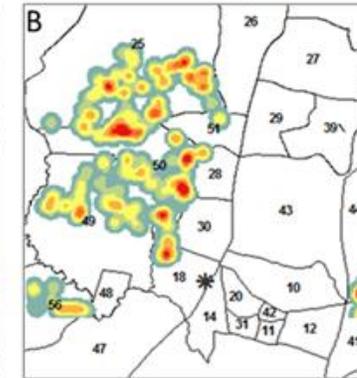
Bed Net Use

2011



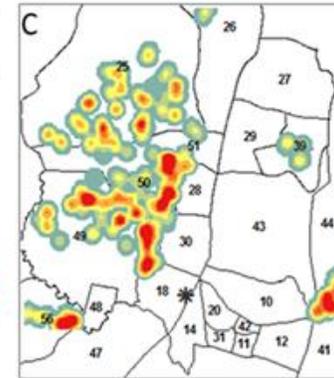
53.8%

2012



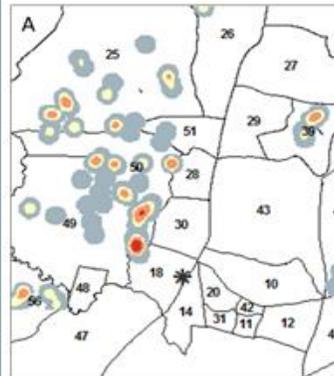
59.1%

2013

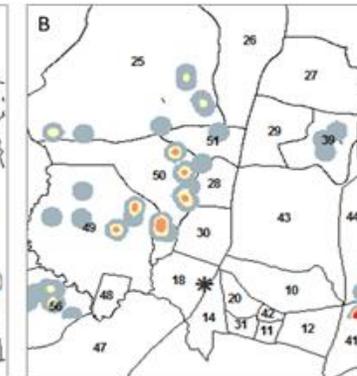


78.6%

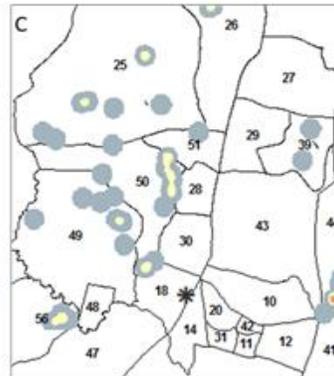
Malaria Prevalence



12.9%

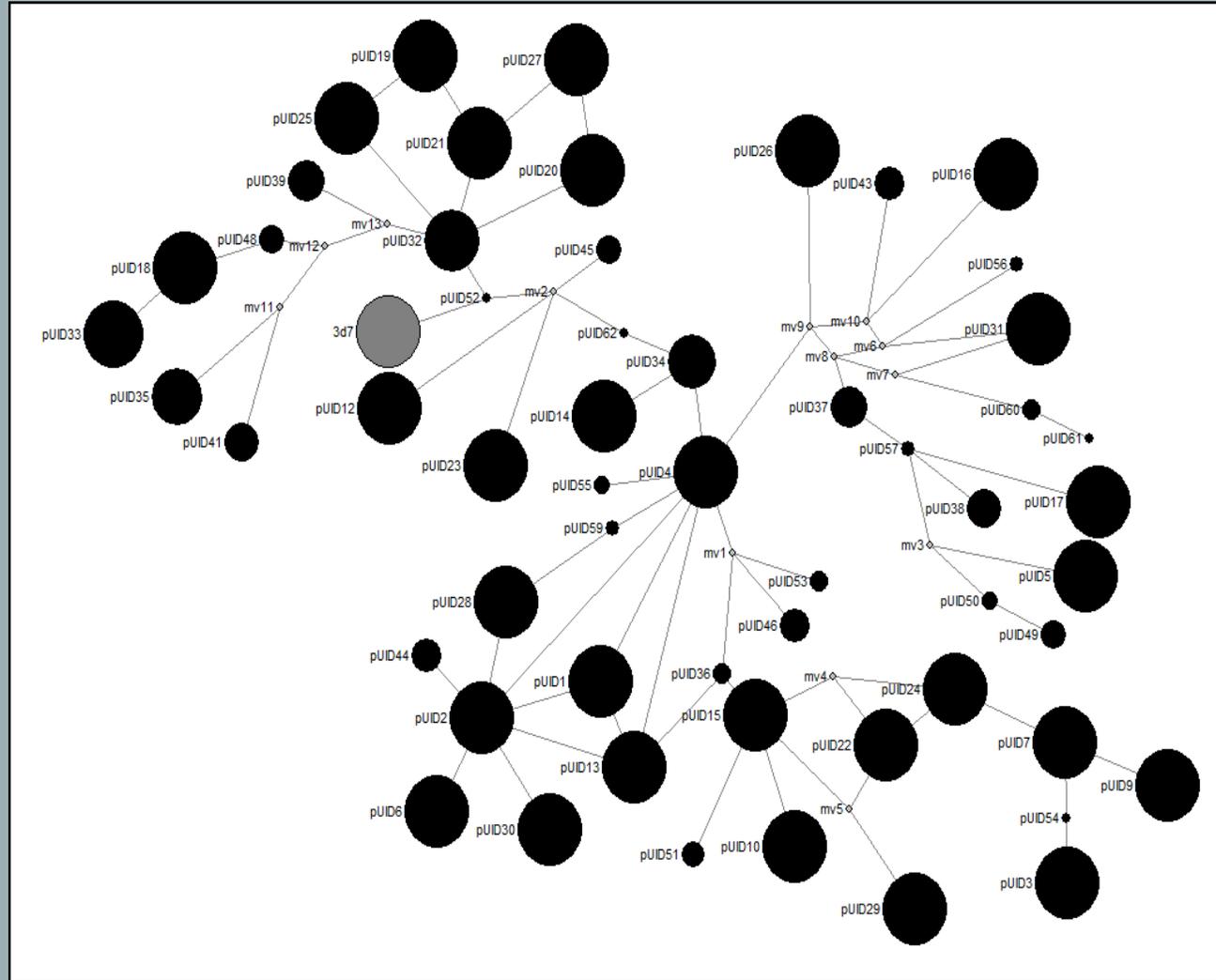


8.0%



5.6%

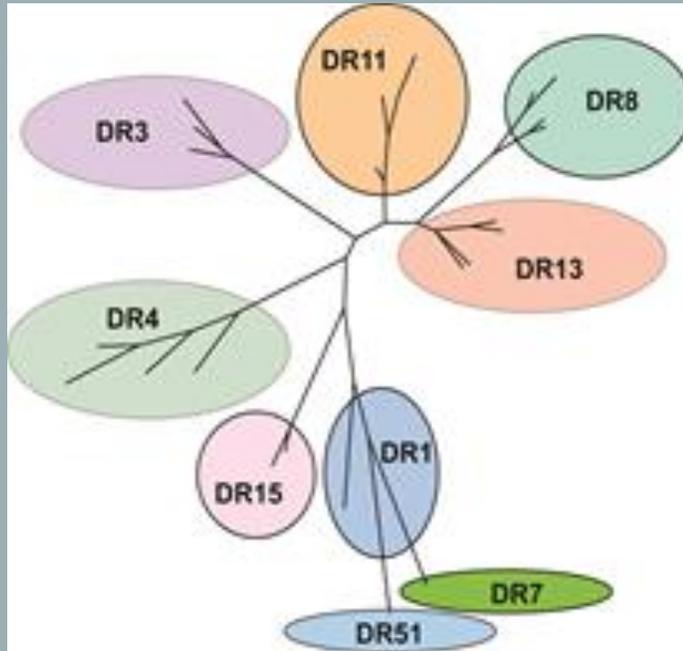
# MALARIA PARASITE DIVERSITY



The figure suggests that *csp* strains in Lilongwe are clustered in several major groups

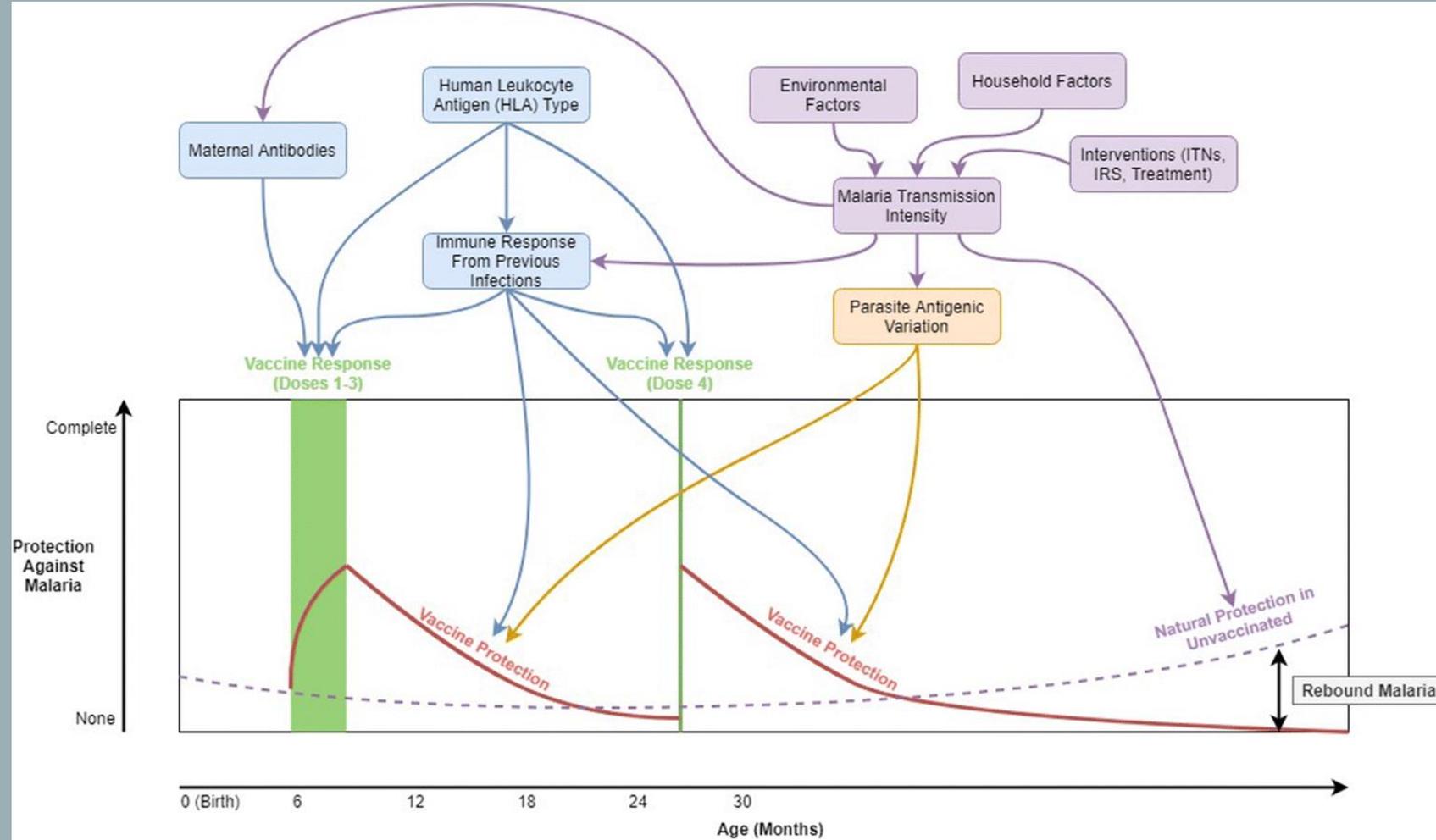
# HUMAN HOST GENETIC DIVERSITY- HLA TYPE

## Distribution of HLA Class II alleles in Malawian women



Supertype	Frequency Allele 1	%	Frequency Allele2	%
DR1	13	11.1		
DR3	24	20.5	8	7.7
DR4	3	2.6	3	2.9
DR7	5	4.4	15	14.4
DR8			10	9.6
DR9			8	7.7
DR10			12	11.5
DR11	27	23.1	15	14.4
DR12	5	4.3	6	5.8
DR13	5	4.3	25	24
DR15	35	29.9	2	1.9
Total	117	100	104	100%

# IMPACTS OF ECOLOGY, PARASITE ANTIGENIC VARIATION, AND HUMAN GENETICS ON RTS,S/AS01E MALARIA VACCINE EFFICACY



# SUMMARY

- There is growing evidence that both genetic variation in the parasite and variation of human host genetic factors affect RTS,S vaccine efficacy.
- These genetic factors may be interacting in complex ways to produce variation in the natural and vaccine-induced immune responses that protect against malaria.
- Transmission intensity may have a role pre- and post-vaccination in modulating immune responses to the vaccine.





## Spatial Health Research Group

University of North Carolina at Chapel Hill

Home  
Spatial Health Research Group  
Research Team  
Projects  
Prospective Students  
Links

**Contact Information**  
Dr. Michael Emch  
UNC-Chapel Hill  
C83220  
Chapel Hill, NC 27599  
All pictures courtesy of  
Research Team members

### Spatial Health Research Group



**Check us out on Twitter:** <https://twitter.com/SpatialHealth>

**The Spatial Health Research Group at the University of North Carolina at Chapel Hill** is led by Dr. Michael Emch. Research activities focus on exploring spatio-temporal patterns of disease, primarily infectious diseases of the developing world. Disease patterns are studied using a holistic approach by investigating the role of social, natural, and built environments in disease occurrence in different places and populations. Diverse statistical and spatial analytical methods are informed by theory from the fields of medical geography, epidemiology, ecology, and others. These theories and methods are used to examine topics such as how social connectivity contributes to disease incidence, the role of population-environment drivers in viral evolution, and using environmental indicators to predict disease outbreaks.



### Funding

National Institute Of Allergies And Infectious Disease, National Institutes Of Health (NIAID), 1R01-AI137410-01.

NSF 20-585 Ecology and Evolution of Infectious Diseases, Award # DEB-210974.

MAL55 Phase III clinical trial for malaria vaccine, PATH Malaria Vaccine Initiative.

National Science Foundation, Award # BCS-0717688