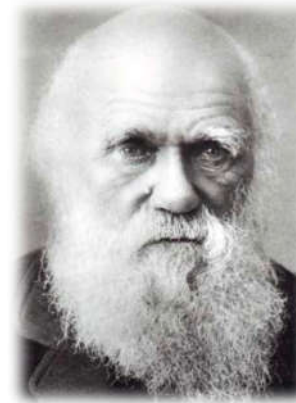


Molecular detective

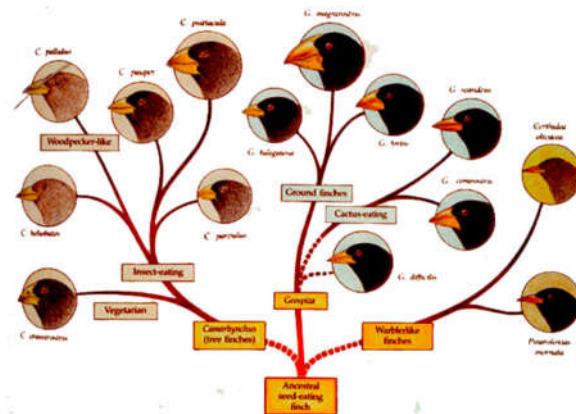
How genetic information help us to investigate disease outbreaks

Natapong Jupatanakul
Medical Molecular Biology Research Unit, BIOTEC
natapong.jup@biotec.or.th

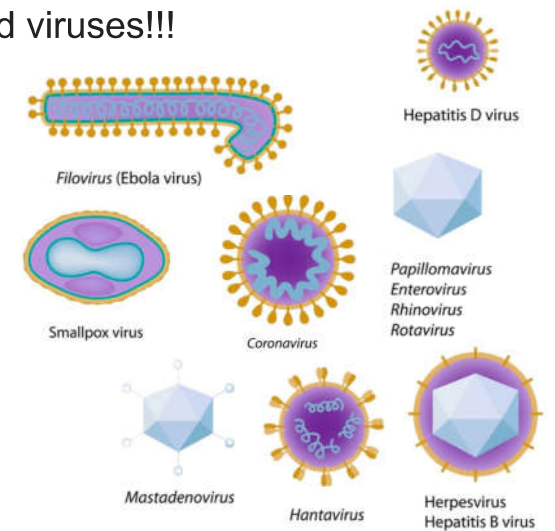
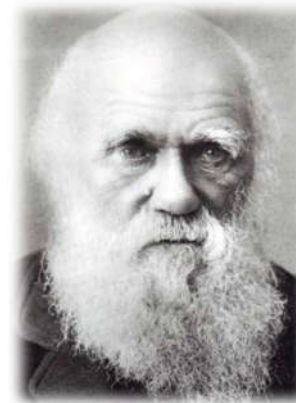
Darwin's tree of life



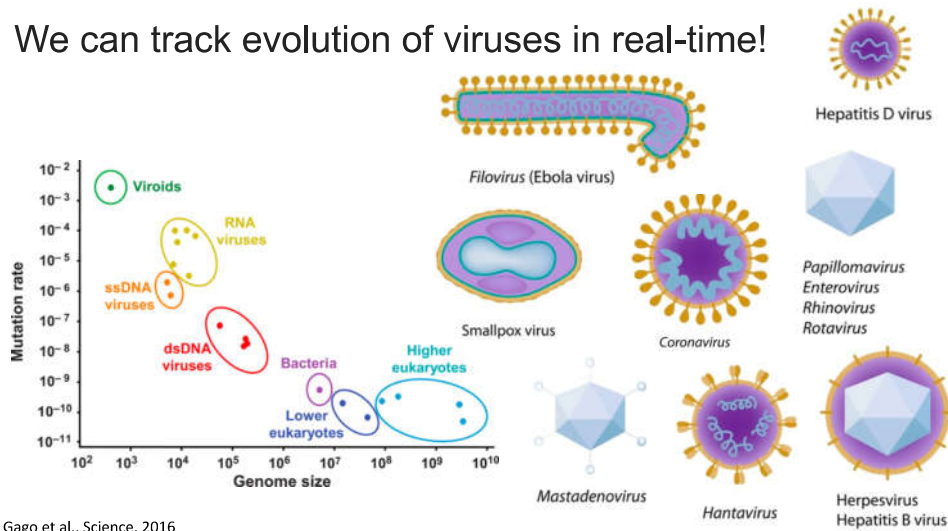
Darwin's theory of natural selection



Darwin would have loved viruses!!!

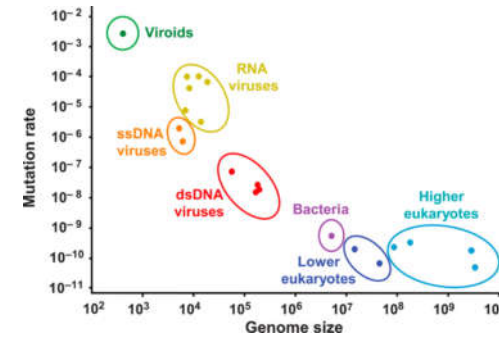


We can track evolution of viruses in real-time!



Gago et al., Science. 2016

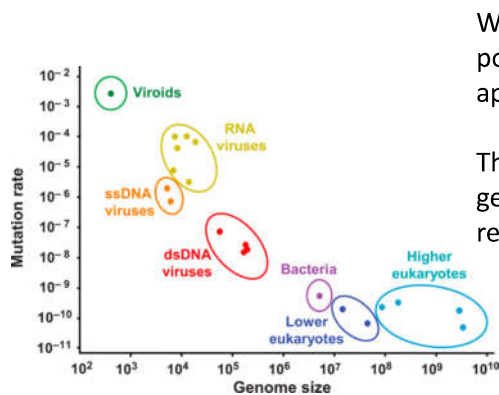
Rapid evolution of viruses is due to:



Gago et al., Science. 2016

- Short generation time
- Large number of progeny
- High mutation rates
- Constantly exposed to selective pressure

Viruses has high mutation rates

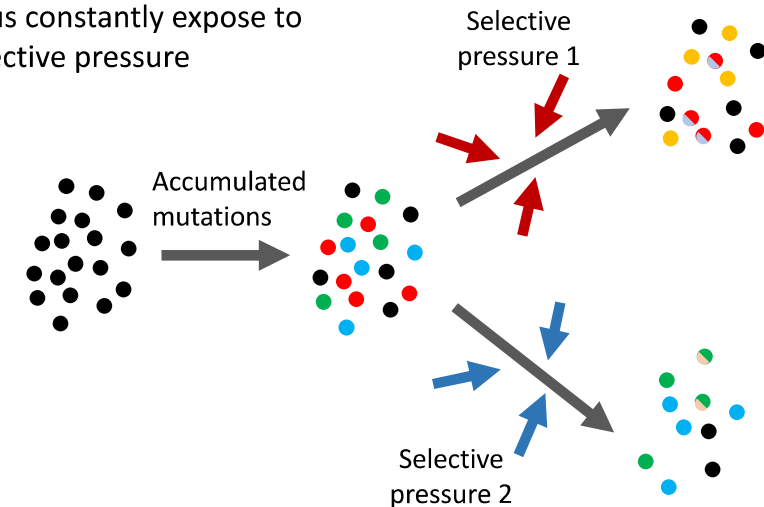


Gago et al., Science. 2016

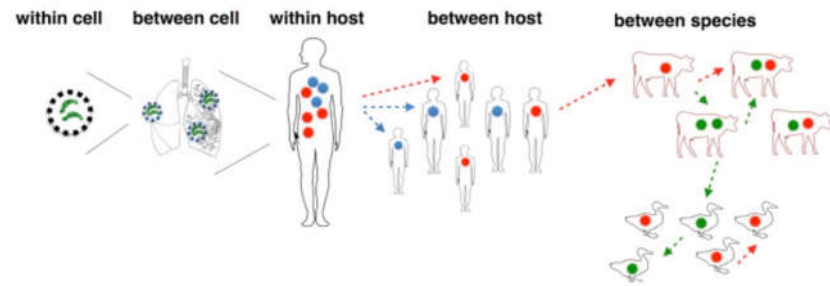
Without proofreading activity, viral RNA polymerase has high error rates at approximately one in 10^4 bp

This means that the RNA virus with 10 kb genome will have one mutation every replication

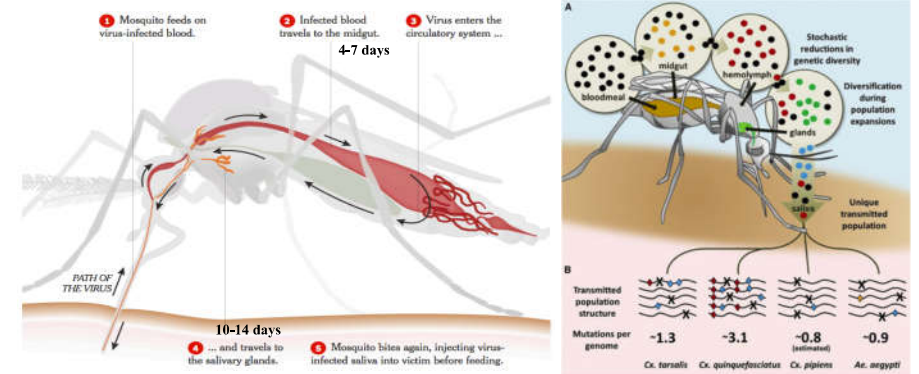
Virus constantly expose to selective pressure



Survival of the fittest

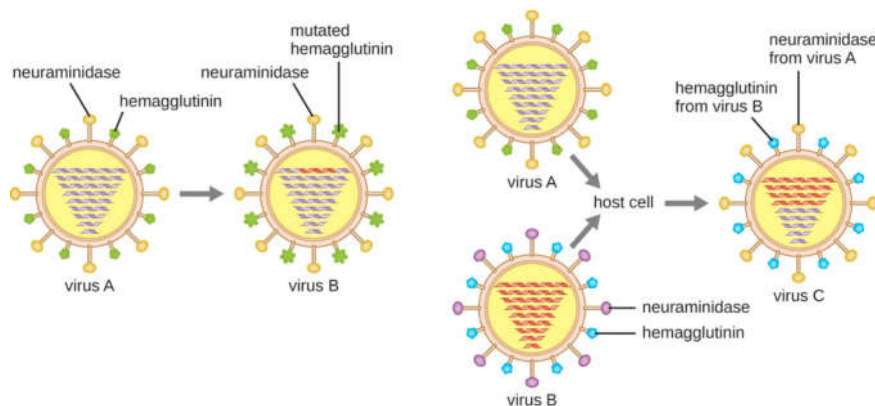


Virus replication in mosquito serve as environmental niches



Grubaugh et al., 2016

Antigenic drift & antigenic shift



We constantly face problems from rapid viral evolutions

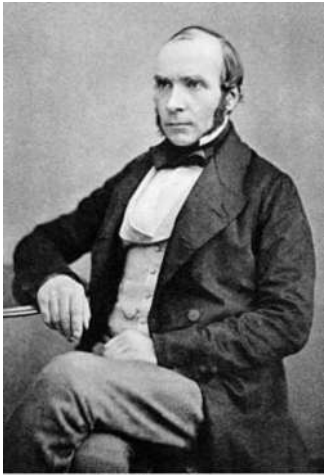
Anti-retroviral drug resistance in HIV



Constant appearance and reappearance of devastating viruses



Flu vaccine requires redesign every year, and the vaccine might not even be effective against circulating strains



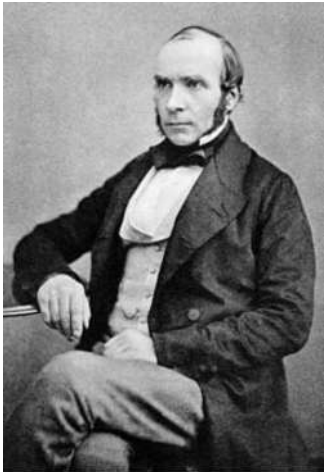
John Snow

John Snow

The World's first epidemiologist

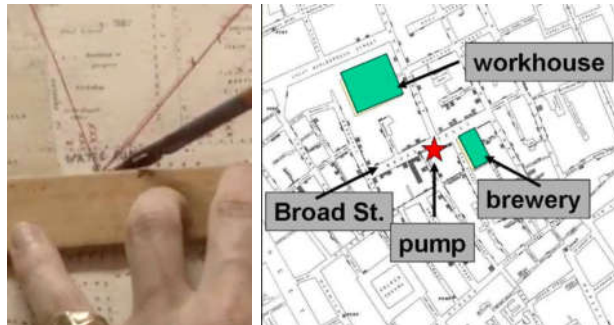


is the study of the **distribution and determinants of health-related states or events (including disease)**, and the application of this study to the control of diseases and other health problems.



John Snow

Investigation of cholera outbreak in Soho, London, in 1854
Miasma theory: diseases caused by bad air
By talking to local residents, he identified the source of the outbreak as the public water pump on Broad Street



Surveillance and descriptive

examining the distribution of disease in a population, and observing the basic features of its distribution

Analytical studies

investigating a hypothesis about the cause of disease by studying how exposures relate to disease

Surveillance and descriptive

Ebola outbreaks
in West Africa

Analytical studies

Chikungunya outbreak
in La Réunion

Plasmodium falciparum
genome analyses

Epidemiology helps us to:

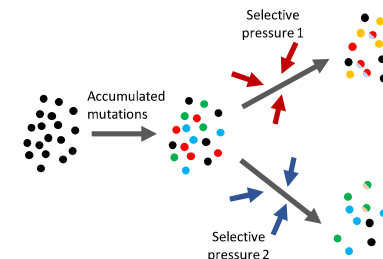
- determine, describe, and report on the natural course of disease, health, disability, injury, and death
- determine the characteristics of the agent or causative factors as well as the mode of transmission
- determine contributing and virulence factors

Epidemiology reduces public health burden by:

- provide a basis for developing disease control and prevention measures for groups at risk
- develop measures to prevent or control disease

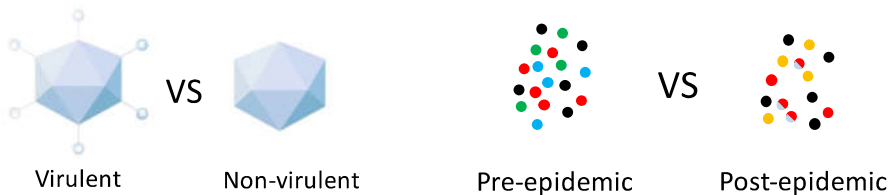
Genetic information facilitate epidemiological investigation

- Genetic mutation that accumulate over time provides a history record of infectious disease outbreaks



Genetic information facilitate epidemiological investigation

- Comparative bioinformatics analyses help identifying key events (evolution points) that lead to epidemic of the outbreaks

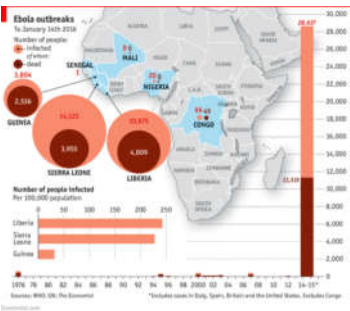


Example Case I Molecular Evidence of Sexual Transmission of Ebola virus

Ebola outbreaks

Year(s)	Country	Ebola subtype	Reported number of human cases	number (%) of deaths among cases	Situation
May - July 2017	Democratic Republic of the Congo	Ebola virus	8	4 (50%)	Outbreak occurred in the Likati health zone of the province of Bas Uele. The response faced challenging logistical obstacles, including the remoteness of the area and limited services. Mobile diagnostic laboratories provided testing of samples in the affected areas.
August-November 2014	Democratic Republic of the Congo	Ebola virus	66	49 (74%)	Outbreak occurred in multiple villages in the Democratic Republic of the Congo. The outbreak was unrelated to the outbreak of Ebola in West Africa. ¹²
March 2014-2016	Multiple countries	Ebola virus	28,616	11,310	Outbreak primarily in Guinea, Liberia and Sierra Leone. Thirty-six confirmed cases were reported from Italy, Mali, Nigeria, Senegal, Spain, the United Kingdom, and the United States. ¹³ A further 24 Ebola-confirmed patients were transported to France, Germany, Italy, the Netherlands, the United Kingdom, the United States, Norway, Spain, and Switzerland. ¹⁴
November 2012-January 2013	Uganda	Sudan virus	6*	3* (50%)	Outbreak occurred in the Luvuvu District. CDC assisted the Ministry of Health in the epidemiologic and diagnostic aspects of the outbreak. Testing of samples by CDC's Viral Special Pathogens Branch occurred at UVR in Entebbe. ¹⁵
June-November 2012	Democratic Republic of the Congo	Bundibugyo virus	36*	13* (36.1%)	Outbreak occurred in DRC's Province Orientale. Laboratory support was provided through CDC and the Public Health Agency of Canada (PHAC)'s field laboratory in Itano, as well as through the CDC/UVR lab in Uganda. The outbreak in DRC had no epidemiologic link to the near contemporaneous Ebola outbreak in the Kibale district of Uganda. ¹⁶

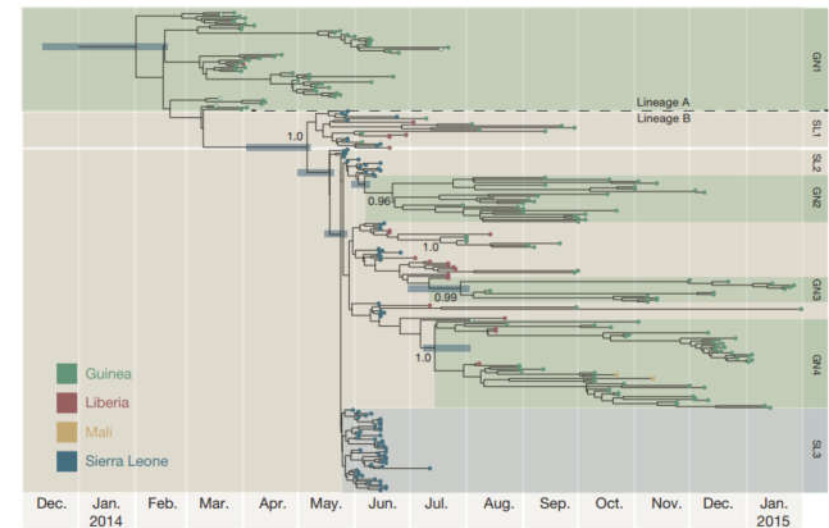
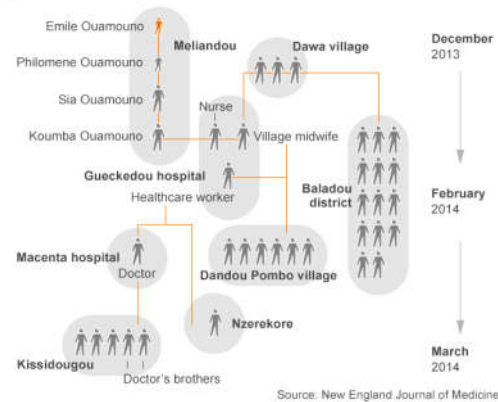
WHO reports



Ebola outbreaks

Aspect	Previous Outbreaks	West Africa 2014-2015
Number of cases	Typically 100-200	~25,000
Number of responding countries, per outbreak	Usually 1	3 (plus ~25 imported)
Duration	2-4 months	> 1 year
Exported cases outside area	Extremely rare	Frequent
Community cooperation	Occasional resistance	Frequent resistance
Organizations responding, per outbreak	5-10	> 100
Urban areas	Spared	Heavily involved
Number of Ebola treatment units employed, per outbreak	Typically 1-2	> 50
Number of diagnostic labs involved in response, per outbreak	Typically 1-2	> 50
Cost of response, per outbreak	< \$5 million	Nearing \$1 billion

The Ebola Epidemic in West Africa: Proceedings of a Workshop, National Academies of Sciences



Carroll et al., Nature, 2015

The NEW ENGLAND JOURNAL of MEDICINE

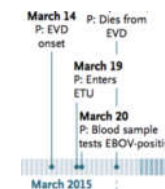
BRIEF REPORT

Molecular Evidence of Sexual Transmission of Ebola Virus

S.E. Mate, J.R. Kugelman, T.G. Nyenswah, J.T. Ladner, M.R. Wiley,
T. Cordier-Lassalle, A. Christie, G.P. Schroth, S.M. Gross, G.J. Davies-Wayne,
S.A. Shinde, R. Murugan, S.B. Sieh, M. Badio, L. Fakoli, F. Taweh, E. de Wit,
N. van Doremalen, V.J. Munster, J. Pettitt, K. Prieto, B.W. Humrighouse,
U. Ströher, J.W. DiClaro, L.E. Hensley, R.J. Schoepp, D. Safronetz, J. Fair,
J.H. Kuhn, D.J. Blackley, A.S. Laney, D.E. Williams, T. Lo, A. Gasasira, S.T. Nichol,
P. Formenty, F.N. Kateh, K.M. De Cock, F. Bolay, M. Sanchez-Lockhart,
and G. Palacios

History and Epidemiology

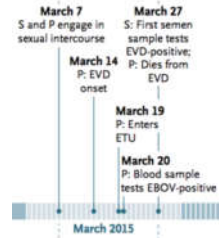
- On March 20, 2015, a 44-year-old woman from Montserrado County, Liberia, was confirmed to have EVD.
- Blood samples from the patient were confirmed to be positive for EBOV RNA.
- The patient died on March 27, 2015.
- The case investigation did not reveal an immediate source of infection, such as contact with patients with acute EVD.



Mate et al., 2015

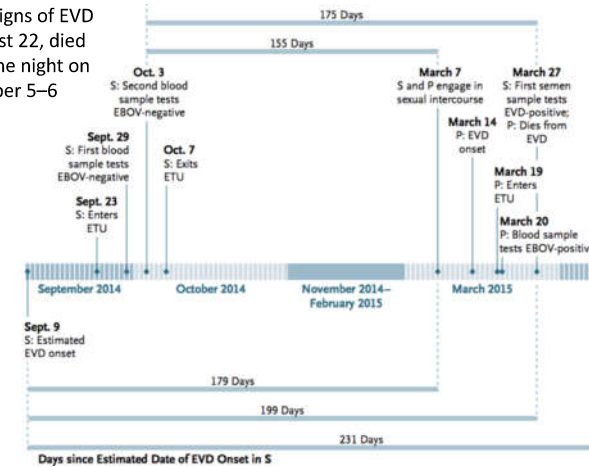
History and Epidemiology

- However, the patient reported that on March 7, 2015, she had had unprotected vaginal intercourse with a male Liberian survivor of EVD.
- Subsequent to the patient's EVD diagnosis, 192 contacts were identified, 11 all of whom were free from clinical signs.



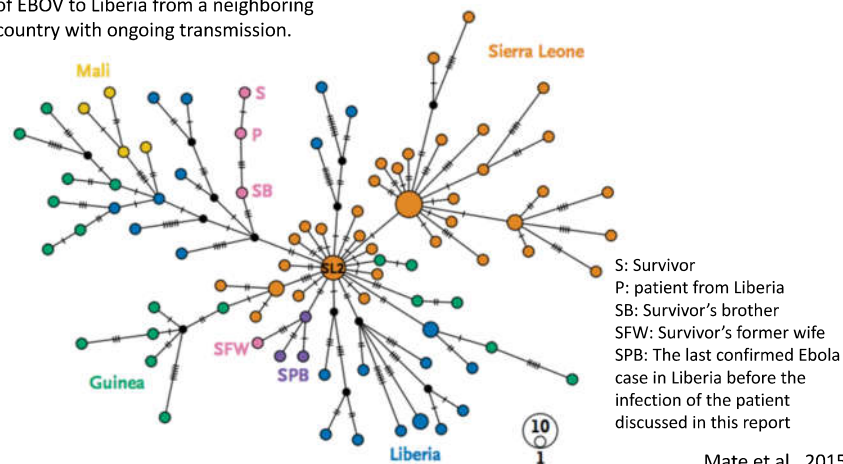
Mate et al., 2015

The survivor's older brother, who presented with clinical signs of EVD on August 22, died during the night on September 5–6

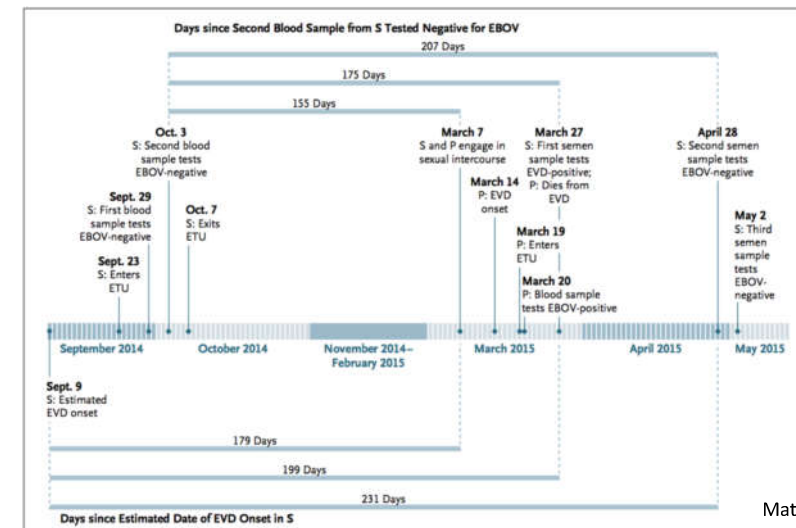


Mate et al., 2015

It is unlikely that the patient was infected owing to an undocumented reintroduction of EBOV to Liberia from a neighboring country with ongoing transmission.



Mate et al., 2015



Mate et al., 2015

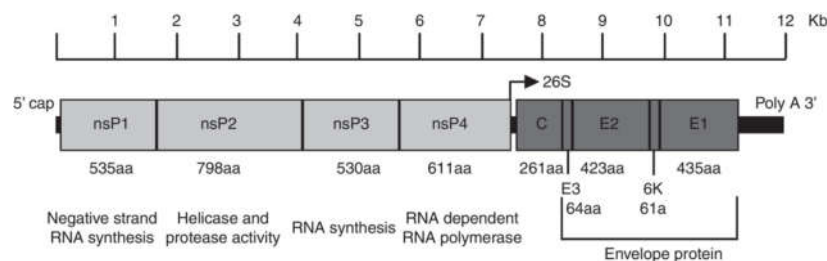
Key points from Case I

- Phylogenetic analysis serve as a tool to trace back infectious disease outbreaks in both time and space
- Genetic information together with bioinformatics analyses strengthen epidemiological findings
- Epidemiological findings leads to better health suggestions and prevention

Example Case II

Evolution of Chikungunya virus during Indian Ocean outbreaks

Chikungunya virus



Mosquito vectors of Chikungunya virus



Aedes aegypti aegypti

- Tropical and subtropical
- Feeds almost exclusively on humans
- Takes multiple bloodmeals within a gonotrophic cycle
- Exploits artificial water containers near houses as larval habitats
- Adult females found mostly inside houses



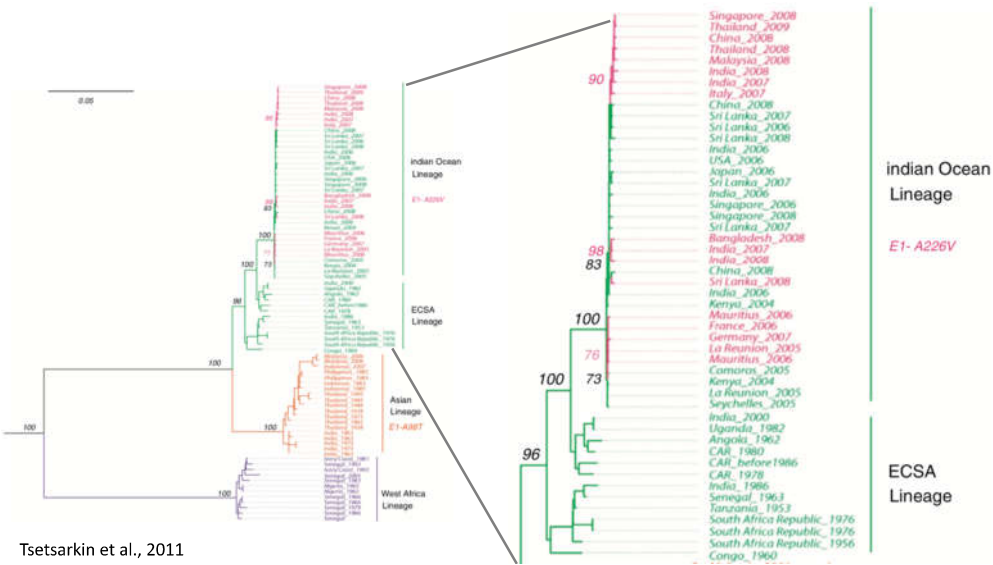
Aedes albopictus

- Invaded tropics, and temperate regions from Asia since 1985
- Feeds opportunistically
- Usually takes a single bloodmeal within a gonotrophic cycle
- Uses artificial and natural larval habitats
- Varied levels of anthrophily and endophily

The case of Chikungunya virus



Weaver and Forester, 2015



Tsetsarkin et al., 2011



Tsetsarkin et al., 2011

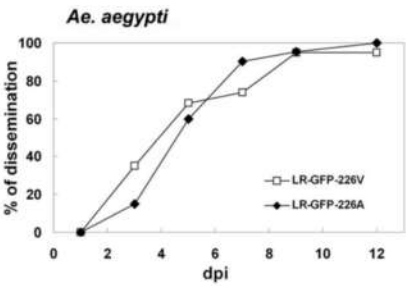
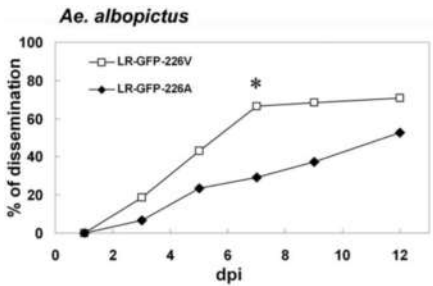
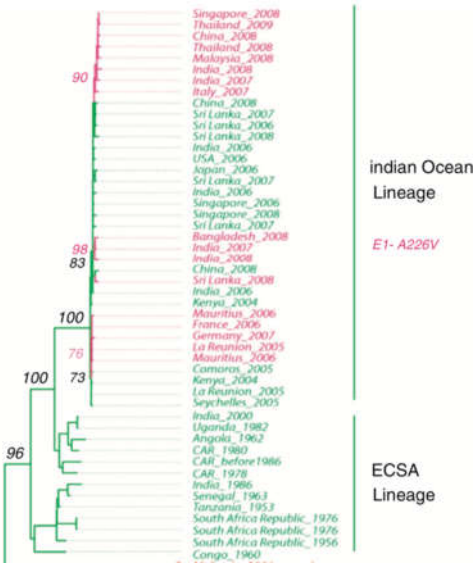
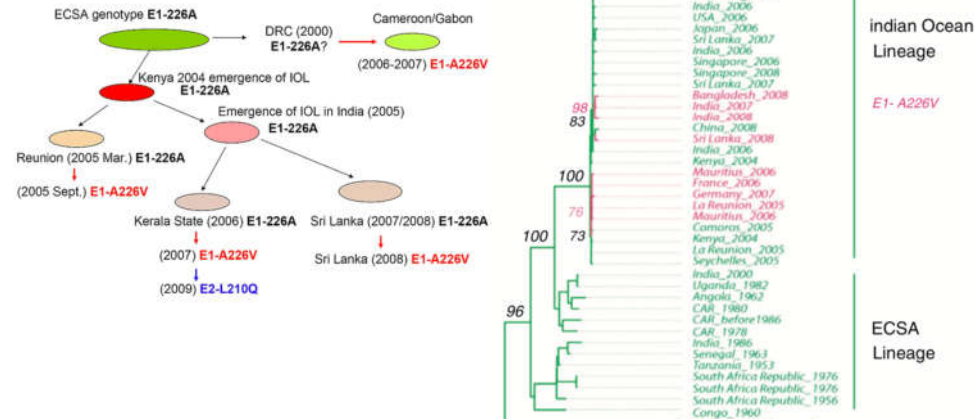


Table 1. Log₁₀OID₅₀/ml for CHIKV in *Ae. albopictus* Mosquitoes

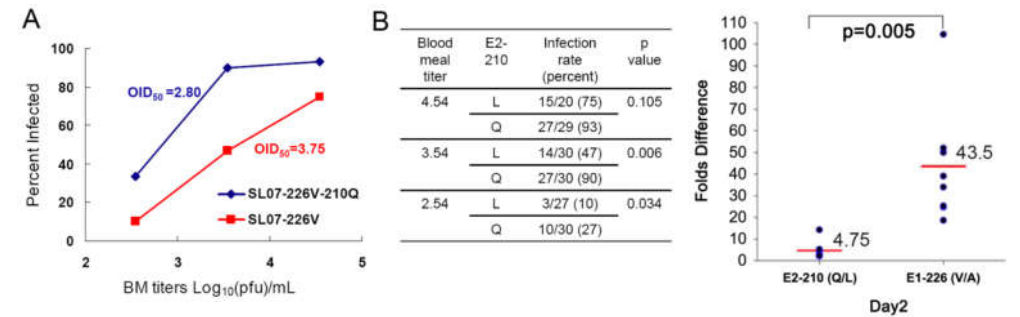
Backbone	Exp ^a	Virus	Mosquitoes Analyzed ^b	Log ₁₀ OID ₅₀ ± CI ₉₅ ^c	P Value
CHIK Reunion	1	LR-GFP-226V	98	<4.22	p<0.01
		LR-GFP-226A	101	5.42 ± 0.29	
	2	LR-GFP-226V	171	3.52 ± 0.28	p<0.01
CHIK 37997		LR-GFP-226A	93	5.48 ± 0.23	
	1	37997-GFP-226A	131	5.20 ± 0.22	p<0.01
		37997-GFP-226V	138	3.31 ± 0.42	
	2	37997-GFP-226A	129	4.90 ± 0.25	p<0.01
		37997-GFP-226V	136	3.06 ± 0.32	

E1 A226 increases CHIKV infectivity in *A. albopictus* but not *A. aegypti*

Tsetsarkin et al., 2007

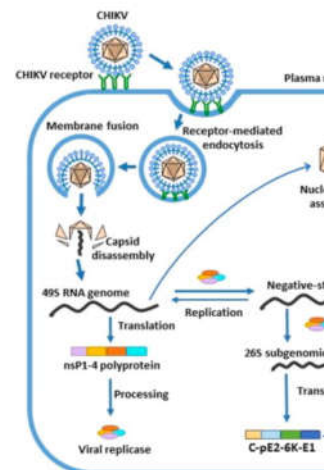
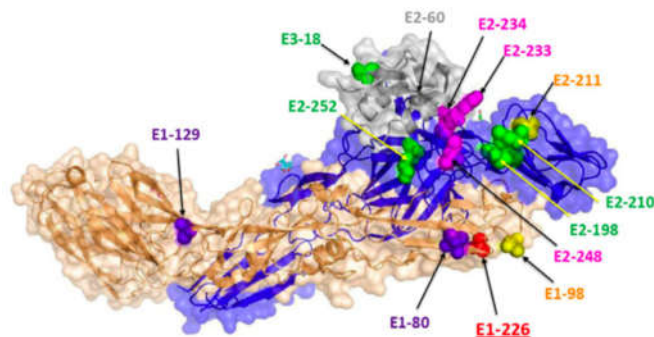


Mutations in CHIKV E proteins increase virus infectivity in *Aedes albopictus* mosquito

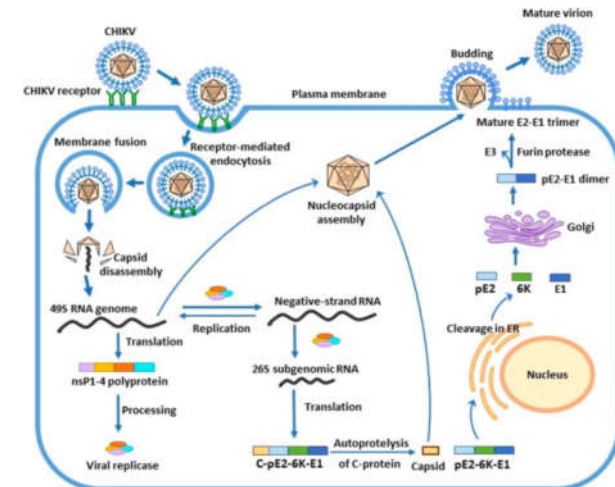


Tsetsarkin and Weaver, 2011

CHIKV E proteins



Weaver and Forester, 2015



Abdelnabi et al., 2015



Aedes aegypti aegypti

- Tropical and subtropical
- Feeds almost exclusively on humans
- Takes multiple bloodmeals within a gonotrophic cycle
- Exploits artificial water containers near houses as larval habitats
- Adult females found mostly inside houses
- Moderately susceptible to CHIKV



Aedes albopictus

- Invaded tropics, and temperate regions from Asia since 1985
- Feeds opportunistically
- Usually takes a single bloodmeal within a gonotrophic cycle
- Uses artificial and natural larval habitats
- Varied levels of anthrophily and endophily
- Moderately to highly susceptible to CHIKV



Weaver and Forester, 2015

What can we get from all these?

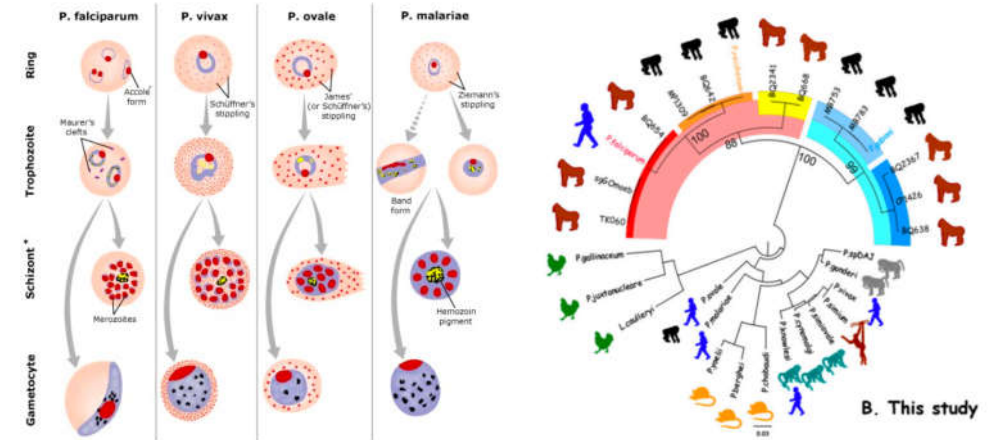
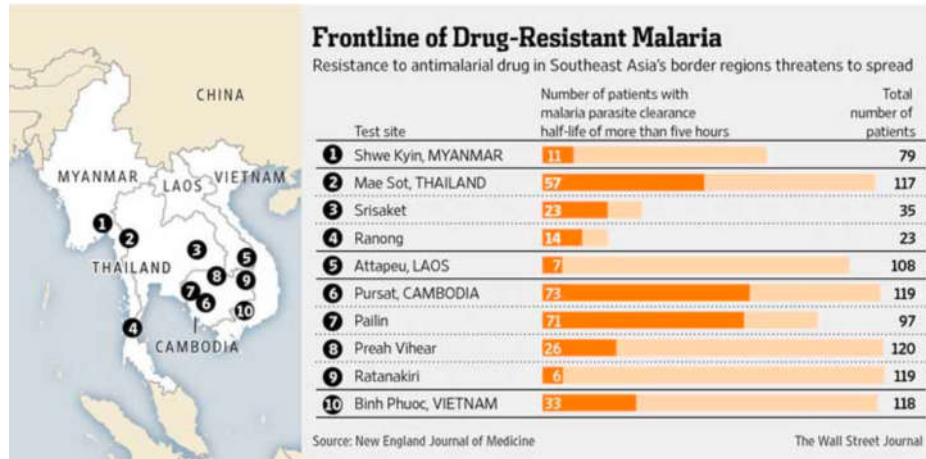
- The control of endemic/epidemic transmission is potentially more feasible, in *A. albopictus* compared to *A. aegypti* because of the less endophilic nature of *A. albopictus* makes it more susceptible to outdoor adulticide spray applications
- A design of live-attenuated CHIKV that cannot be transmitted by mosquito is possible
- Compound that target certain epitopes of the CHIKV E proteins can serve as transmission blocking molecule and exacerbate the CHIKV transmission, especially in *A. albopictus* setting

Key points from the Case II

- Again, molecular information help in explaining epidemiological discoveries
- With proper genetic tools, evolutionary changes that leads to epidemic can be experimentally confirmed, and eventually results in translational impacts

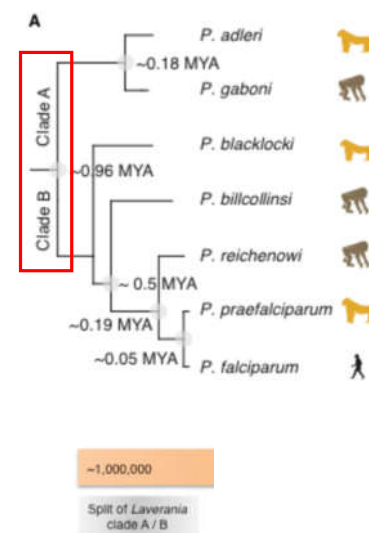
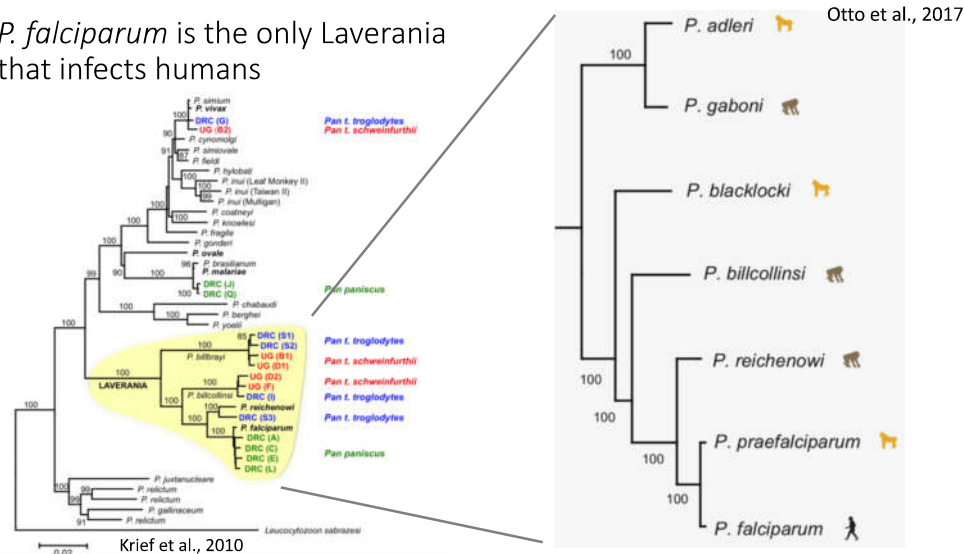
Example Case III

Application of genome scale phylogenetic analyses in *Plasmodium falciparum*



Prugnolle et al., 2009

P. falciparum is the only *Laverania* that infects humans

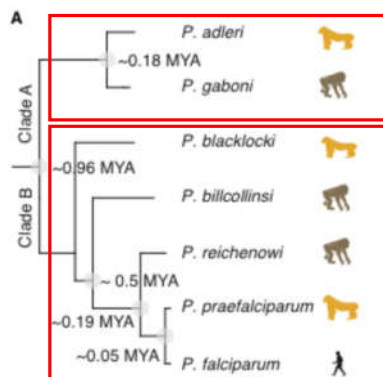


P. falciparum is derived from a group of parasites infecting African Great Apes and known as the *Laverania* subgenus

Chimpanzees: *P. gaboni*, *P. billcollinsi* and *P. reichenowi*

Gorillas: *P. praefalciparum*, *P. blacklocki* and *P. adleri*

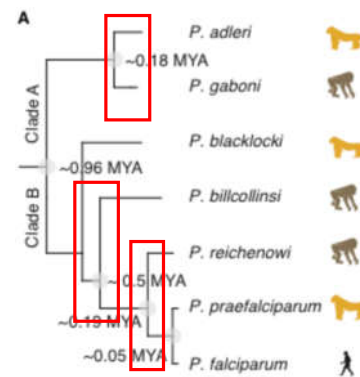
Humans: *P. falciparum* only



Otto et al., 2017

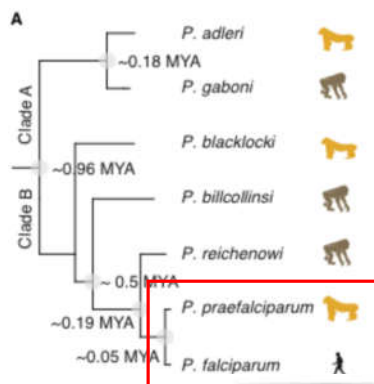
~ 1,000,000 years ago, Laverania divided into two clades marking a key evolutionary split

Possible explanation:
Geographical changes?
Extreme environmental changes?
Extinction and rise of new hominid?



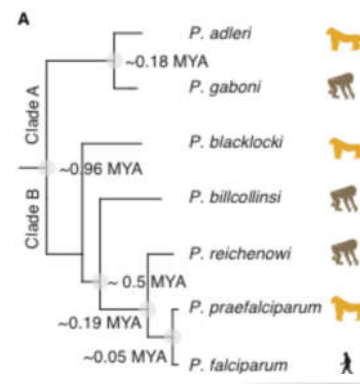
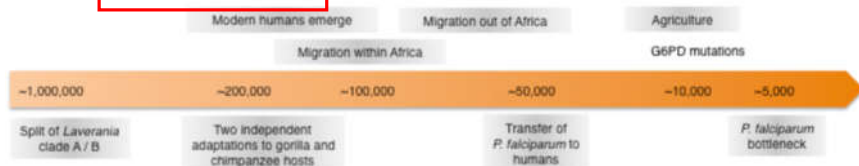
Otto et al., 2017

Subsequent host adaption in both clades

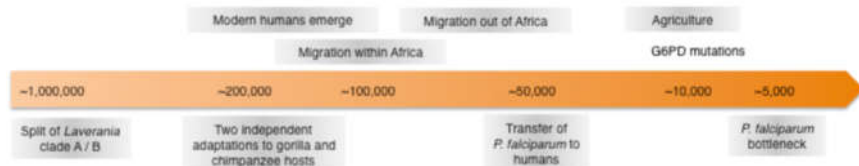
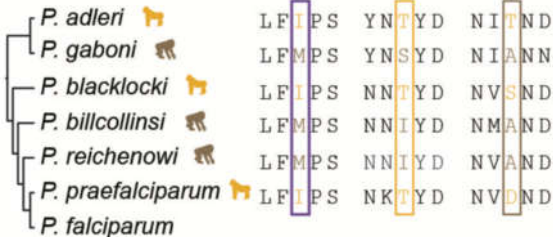


Otto et al., 2017

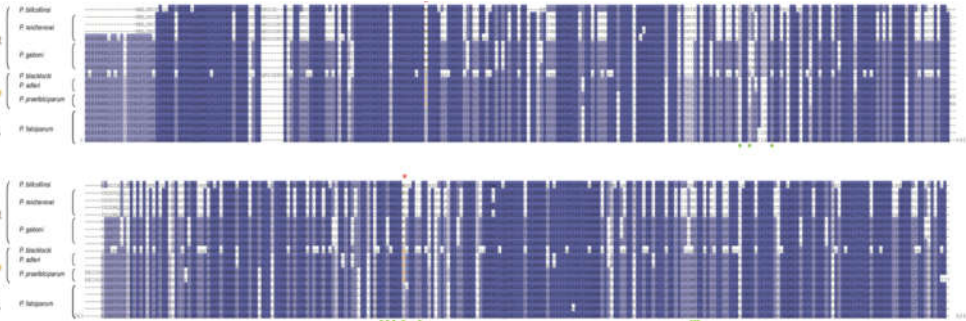
P. falciparum only recently adapt to humans around 50,000 years ago even though modern humans emerged way before that



Otto et al., 2017



Convergence leads to an incorrect phylogenetic tree

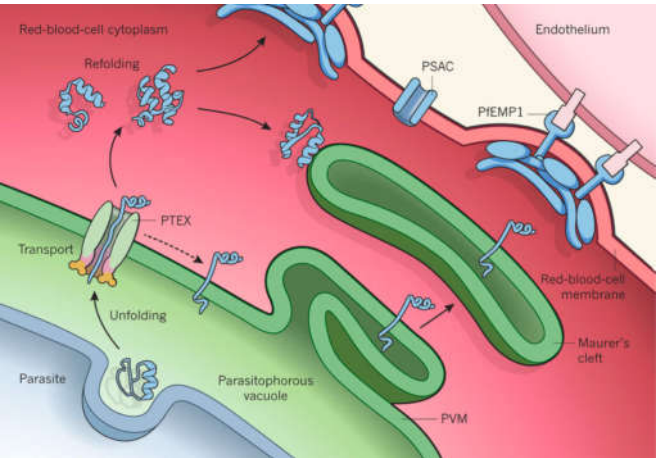
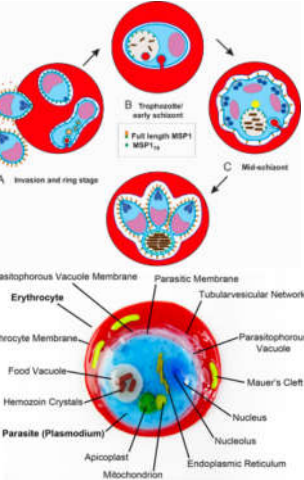


Con

	<i>P. falciparum</i>	<i>P. praefalciparum</i>	<i>P. reichenowi</i>	<i>P. blacklocki</i>	<i>P. billcollinsi</i>	<i>P. gaboni</i>	<i>P. adleri</i>
var	67	112	92	35	43	61	58
rif	186	758	456	326	18	18	20
stevor	37	81	62	46	0	0	11
hyp4	9	2	1	1	0	0	0
hyp5	9	2	0	0	4	2	0
Maurer	13	12	5	4	7	0	0
exp1	8	6	4	3	5	1	1
RESA-like	6	5	2	2	1	3	3
CLAG	5	7	6	7	35	16	24
DBLmsp	1	1	1	1	1	4	7
glycophorin binding	3	4	5	6	1	3	5
MSP7-like	8	8	7	6	4	7	14
Acyl-Co	13	17	17	11	18	16	26

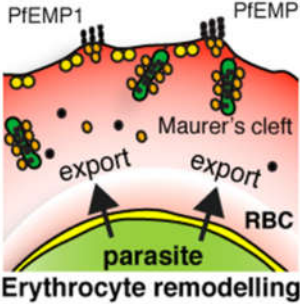
Otto et al., 2017

Dluzewski., 2008



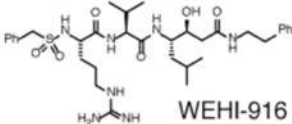
Desai and Miller, 2014

virulent malaria infection

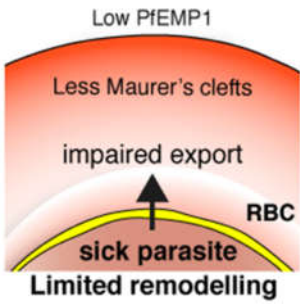


PEXEL cleavage blocked

Plasmeprin V inhibitor



parasite death



Caitlin Sedwick, 2014

Key points from the case III

- Phylogenetic trees of organisms generated from multiple genes reflect evolutionary relationship more accurately as convergence of evolution leads to similarity of certain genes with distinct evolutionary relationship
- Whole genome comparative analyses can help us to identify new drug target, resistant gene, etc.

