

Are all malaria parasites created equal?

YES

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Extreme Inequality: The theory of Clonal *P.falciparum* Populations of Rich & Ayala

- “In clonal organisms, the search for vaccines & drugs is more likely to be successful if preceded by identification of clonal lineages, targeting those that are more pathogenic or ubiquitous”

from “*Is sex better? Parasites say “no”.*” F. Ayala. PNAS 95, 3346-48. 1998

- “In clonal organisms the persistent entity is the *clonal lineage* and the genetic diversity of the species can be captured only by extensive sampling of distinct lineages”

from “*Is sex better? Parasites say “no”.*” F. Ayala. PNAS 95, 3346-48. 1998

“A clonal population structure is consistent with physiological sexuality, as it is required in *Plasmodium* to complete the life cycle. What it excludes is the prevalence of genetic sexuality, i.e. recombination between genetically heterogenous haplotypes”

****Plasmodium falciparum* antigenic diversity:evidence of clonal population structure.**

Rich, S.M et al PNAS 94, 13040-13045. 1997

Malaria's Eve: evidence of a recent population bottleneck throughout the world population of *Plasmodium falciparum*.

Rich et al PNAS 95, 4425-4430. 1998

- If we wish to call drug resistant *isolates*, drug resistant *strains* we are free to do so.
- BUT we would gain clarity and analytical insight if instead we called such isolates by the more accurate term- *drug resistant mutants*.
- Selection for resistant mutants will *transiently* decrease population variation

Are current data on population genetics of *P.falciparum* compatible with clonality?

- “a selective sweep simultaneously affecting all chromosomes could happen if the population structure of *P.falciparum* were effectively clonal” *Malaria’s Eve*, Rich et al 1998.
- **However this has not happened.** Selective sweeps associated with folate inhibitor resistance are confined to 70kb of DNA *around the dhfr gene*. High density microsatellite chromosome marking shows recombination has narrowed the margins of the drug resistance mediated selective sweep*.
- This is **incompatible** with effective population clonality for *P.falciparum*.

Reduced variation around drug-resistant dhfr alleles in African *Plasmodium falciparum. Pierce, R., Malisa, A., Kachur, S.P., Barnes, K., Sharp, B. and Roper, C. (2005). *Mol. Biol. & Evol.* 22, 1834-44.

Can strains exist in genetically sexual *P.falciparum* populations?

- The evidence against clonal lineages of *P.falciparum* in all except ‘founder effect’ situations is strong.
- If recombination is frequent, albeit proportional to transmission intensity, can strains exist in *P.falciparum*?

Strain Theory

that Pf strains exist because; “*immune selection on polymorphic determinants causes pathogen populations to self-organise spontaneously into discrete antigenic types that may either be maintained over long periods of time or undergo cyclical or chaotic fluctuations*”.

- *The maintainance of strain structure in populations of recombining infectious agents.* (1996) Gupta, Maiden, Feavers, Nee, May & Anderson. *Nature Medicine*.2, 437-42 1996.
- *Population Structure of Pathogens: The Role of Immune Selection.* (1999). Supta & Anderson *Parasitology Today* 15, 497-501.

Assumptions of Strain Theory

1. Malaria in humans is controlled by strain-specific immunity.
2. The multiple strains present in endemic areas are independent.
3. Individuals develop lifelong variant specific immunity.
4. The basic case reproduction number for each strain is low.

Unique PfEMP-1 repertoires?

“ The emergence of discrete, stable antigenic types through immune selection underpins the strain theory of malaria transmission by proposing specifically that the human immune response against PfEMP-1 can cause the population to self-organise into antigenic types characterized by unique PfEMP-1 repertoires.....

However it remains to be determined by molecular methods whether isolates from individuals with different clinical syndromes are characterized by unique PfEMP-1 repertoires”.

Population Structure of Pathogens: The Role of Immune Selection. (1999). Gupta & Anderson *Parasitology Today* 15, 497-501.

Are strains structured by unique combinations of PfEMP-1 genes?

- PfEMP-1 sequences are variable but constrained, and clearly fall into distinct sub-groups.
- The subclasses occur in all isolates tested
- Sub-grouping of *P.falciparum* 3D7 var genes based on sequence analysis of coding and non-coding regions. Lavstsen *et al Malaria J.* 2003. 2.27.

Molecular observations that seem incompatible with unique PfEMP-1 repertoires (1)

The 60 var genes group into essentially 3 families (AB+C) . Parasites causing severe malaria in children express (in RT-PCR assays) a limited, more conserved set of PfEMP-1 than parasites causing uncomplicated malaria in children. Thus limited genetic diversity of PfEMP-1 appears to reflect limited functional diversity

Lavstsen et al POSTER 316C

Molecular observations that seem incompatible with unique PfEMP-1 repertoires (2)

Parasites causing malaria in young children who have not yet acquired immunity, express semi-conserved PfEMP-1 associated with severe disease syndromes (PfEMP-1sm)

Magistrado *et al* POSTER 444C

Molecular observations that seem incompatible with unique PfEMP-1 repertoires (3)

Increased transcription of PfEMP-1 var gene groups A and B, but not C, are associated with severe malaria in young children

Dahlback *et al* POSTER 444C

“Where there are sexually interbreeding organisms the individual genotype is ephemeral; what persists and evolves is the gene pool and a few individuals encompass most of the genetic variability of the species”.

For functional PfEMP1 diversity, perhaps only 1???

from “*Is sex better? Parasites say “no”.*” F. Ayala. PNAS 95, 3346-48. 1998

Molecular observation that seems rather incompatible with unique PfEMP-1 repertoires (4)

Based on the comparison of total var gene diversity in 3 separate *P.falciparum* clones a statistically very robust observation is that;

Intra-clone PfEMP1 diversity > Inter-clone PfEMP-1 diversity

Lavstsen et al. (unpublished but Thomas swears its true)

Molecular evidence shows that isolates from individuals with different clinical syndromes are unlikely to be characterised by unique PfEMP-1 repertoires.

We must conclude that if malaria parasites are not structured into strains by unique repertoires of PfEMP-1 then with respect to this characteristic at least,

all malaria parasites are created equal.