Plasmodium vivax malaria in Madagascar

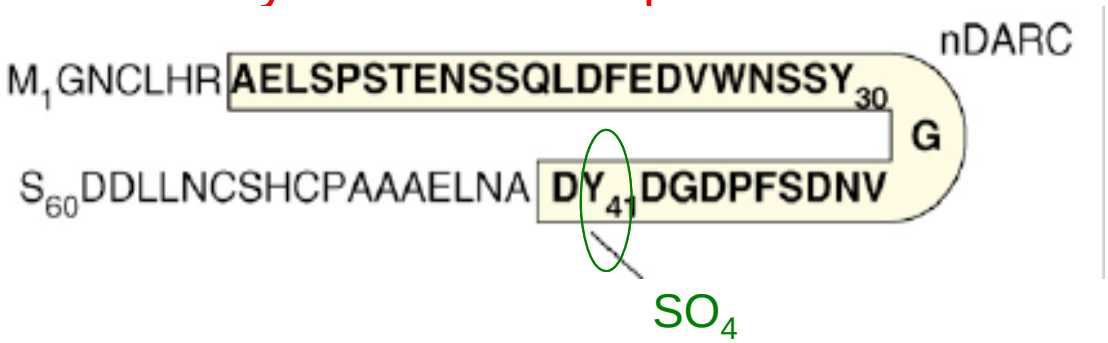
P. vivax infection & clinical malaria in Duffy-negative Malagasy people

Ménard D, Barnadas C et al, Proc Natl Acad Sci U S A. 2010 Mar 30;107(13):5967-71

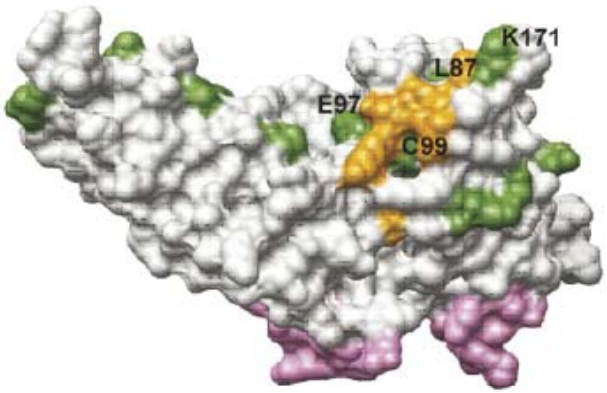
Insights into the possible molecular basis

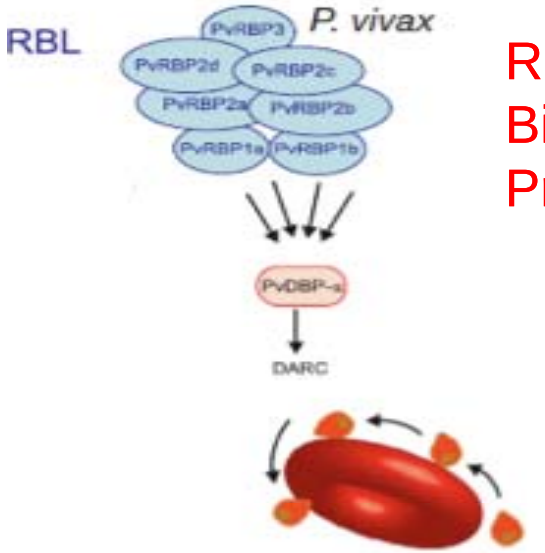
P. vivax interaction with Duffy is required to enable its invasion of human erythrocytes

Duffy/DARC : 10^4 copies/RBC



P. vivax Duffy Binding Protein





Reticulocyte Binding Protein

Assaults on the paradigm: Major role of the Duffy antigen in P. vivax invasion mechanisms

Worldwide distribution of *P. vivax*

From Picot S, 2006

✓ **Ryan et al, 2006 :**
report of anopheles infected by *P. vivax* in Kenya (duffy negative populations)

✓ **Cavasini et al, 2007 :**
report of *P. vivax* PCR positive patients, genotyped as Duffy negative (n=2) in Brazilian Amazon region

Madagascar: cross road

RECENT (only 2300y before present)
Peopled by succession of Asian and African migrations: A

Coexistence of 4 human malaria species *falciparum, vivax, ovale, malariae*

Study population

✓ 8 sentinel sites, 2006-2007

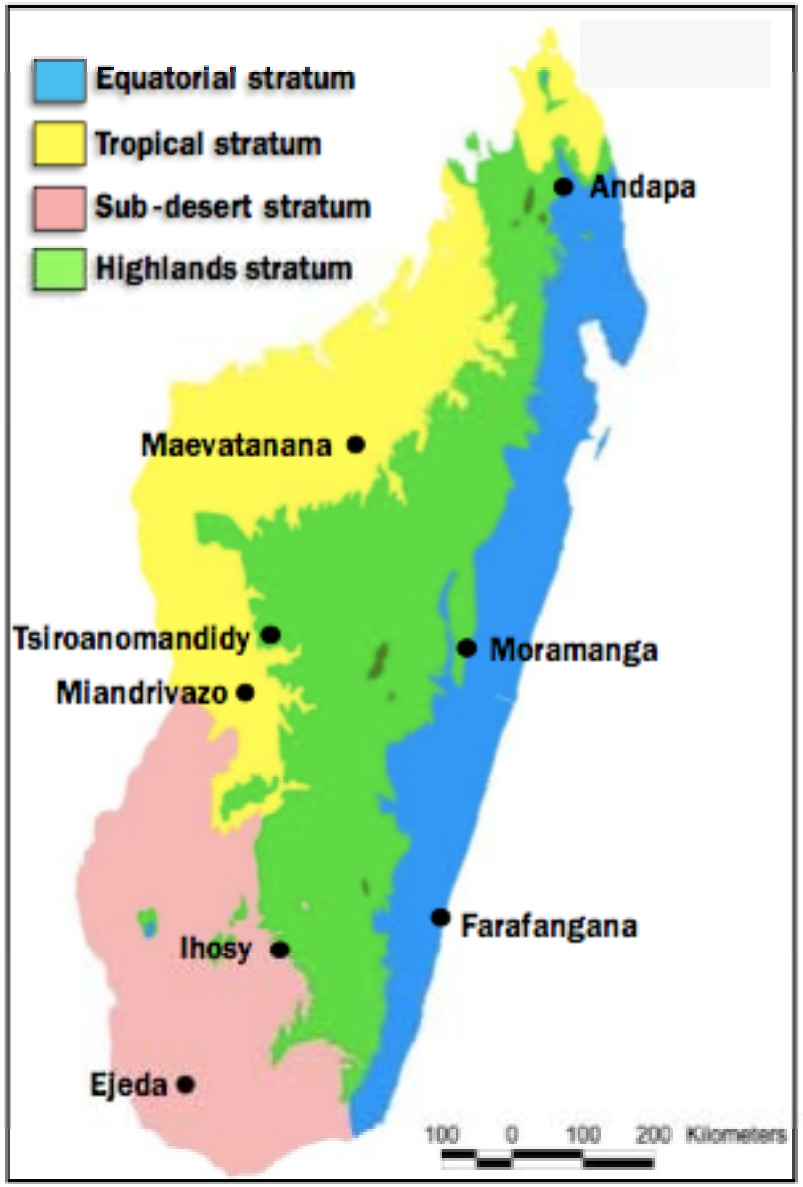
Asymptomatic Schoolchildren
(3-13 y old) (Razakandrainibe et al, 2009)

Malaria Patients /Health Centers
(> 6 mo) (Barnadas et al, 2008)

✓ Malaria diagnosis:
PCR SSU rDNA & microscopy

✓ Duffy genotyping

Duffy -33, promoter +/- (FY^{ES}),
codon 42, FY*A or FY*B;
codon 89, *FY*B* or *FY*X*

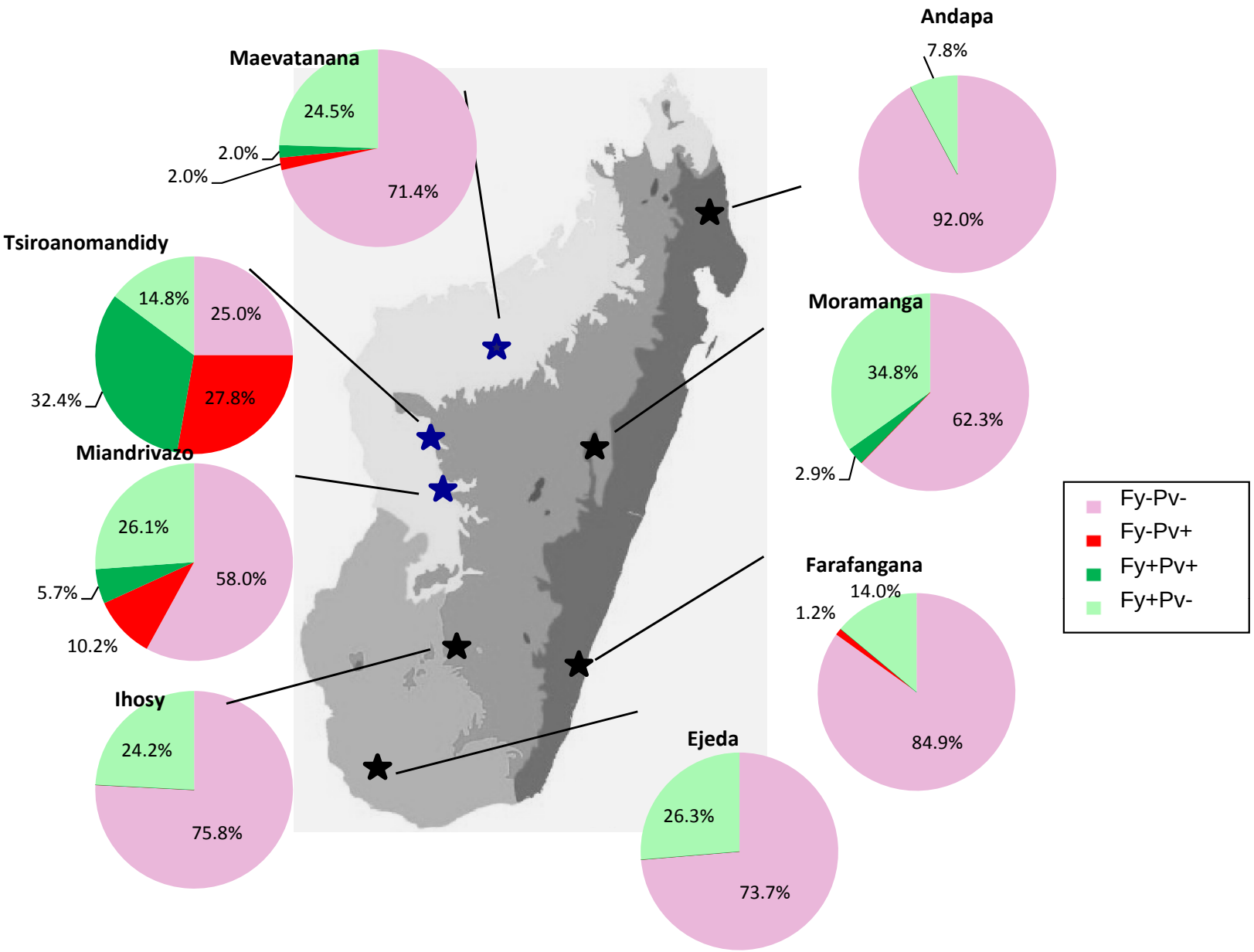


Plasmodium infections and duffy genotype: asymptomatic population (Malagasy schoolchildren)

661/709 (93%) were successfully genotyped

Duffy Phenotype	Fya^+/Fyb^-		Fya^+/Fyb^+	Fya^-/Fyb^+		Fya^-/Fyb^-	Totals
Duffy Genotype	***FY*A/*A***	***FY*A/*B^{ES}***	***FY*A/*B***	***FY*B/*B***	***FY*B/*B^{ES}***	***FY*B^{ES}/*B^{ES}***	
Total population	25 (3.8%)	117 (17.7%)	11 (1.7%)	1 (0.1%)	31 (4.7%)	476 (72.0%)	661
P. vivax infection (prevalence)	2 (8.0%)	33 (28.2%)	5 (45.5%)	-	4 (12.9%)	42 (8.8%)	86 (13.0 %)
Plasmodium sp infection (prevalence)	12 (48.0%)	45 (38.5%)	6 (54.5%)	-	6 (19.4%)	121 (26.7%)	190 (28.7 %)

Duffy genotyping was based on the SNPs Duffy -33, promoter +/-; codon 42, FY*A or FY*B; codon 89, *FY*B* or *FY*X*) using a post-PCR LDR-FMA (ligase detection reaction-fluorescent microsphere assay).
Plasmodium species diagnosis was based on species polymorphisms of the small-subunit ribosomal (SSU) rRNA gene.



Asymptomatic children: release of merozoites from the liver?
True intraerythrocytic infection?

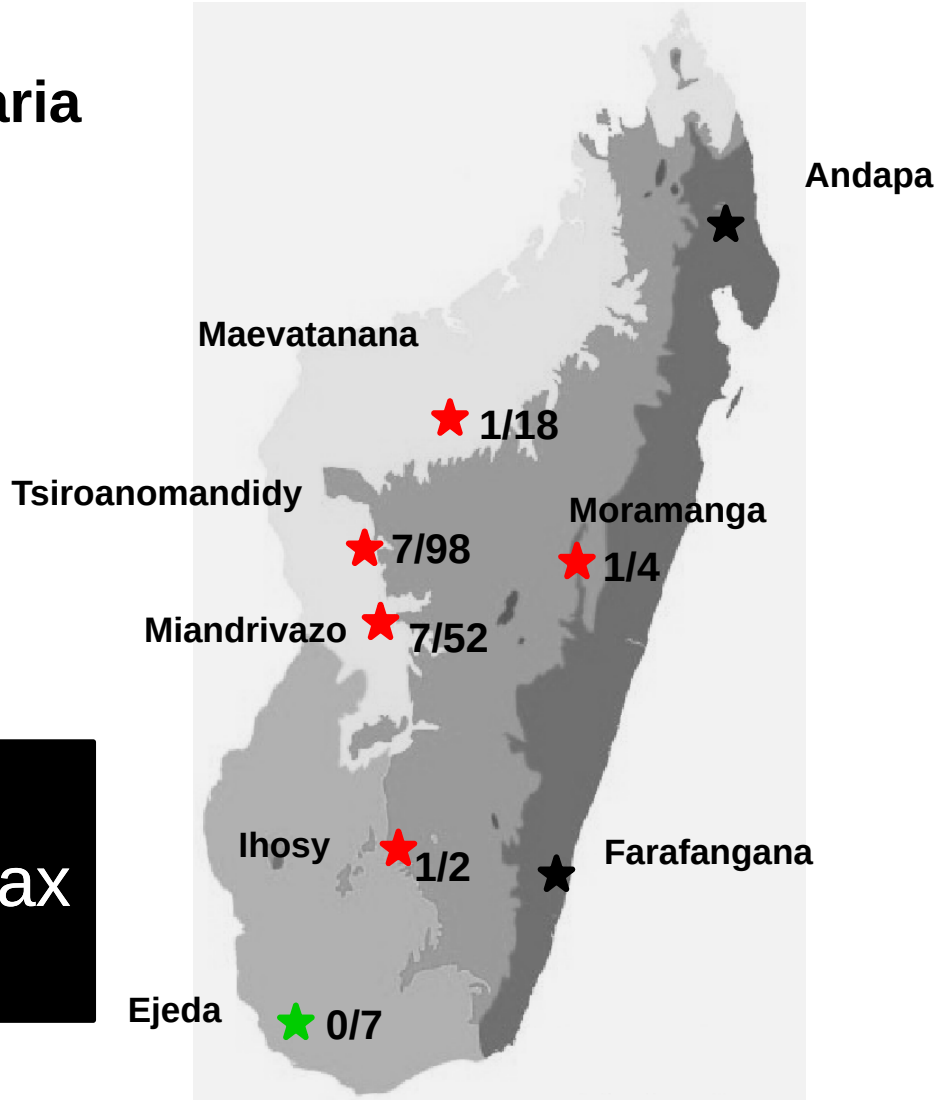
Plasmodium infections and duffy genotype: clinical malaria

183 *P. vivax*-positive infections in malaria patients:

- 150 *P. vivax* mono-infections
  9 in Duffy-neg

- 33 *P. vivax/P. falciparum* infections:
  8 in Duffy-neg

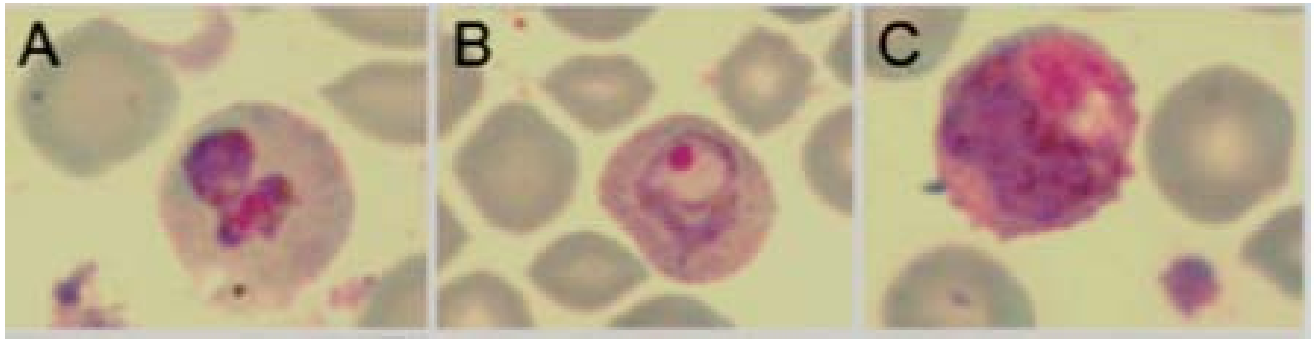
Evidence of *vivax* clinical malaria
17/183 malaria patients with P. vivax are Duffy-neg



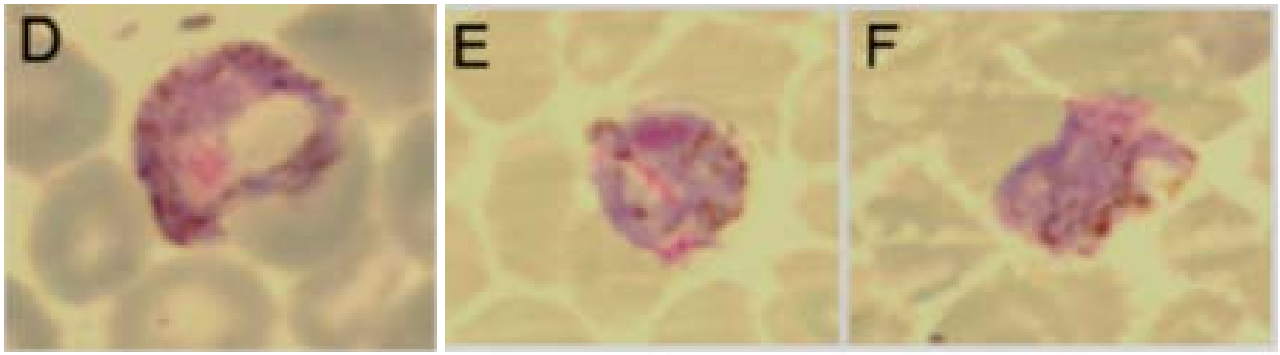
Microscopy evidences?

P. vivax intra-erythrocytic infection in Duffy negative patients: microscopy

(PCR confirmation and exclusion of *P. ovale, P. malariae)*



- genotyped as Duffy-negative ($FY^*B^{ES}/^*B^{ES}$), 4 year old female patient, Tsiroanomandidy,
- microscopy: mixed infection *P. vivax* (3040 infected cells/µl) - *P. falciparum* (980 infected cells/µl).
- PCR confirmation



- genotyped as Duffy-negative (FY*BES/*BES), 3 year old female patient, Moramanga,
- microscopy: P. vivax (3368 parasites infected cells/µl).
- PCR confirmation

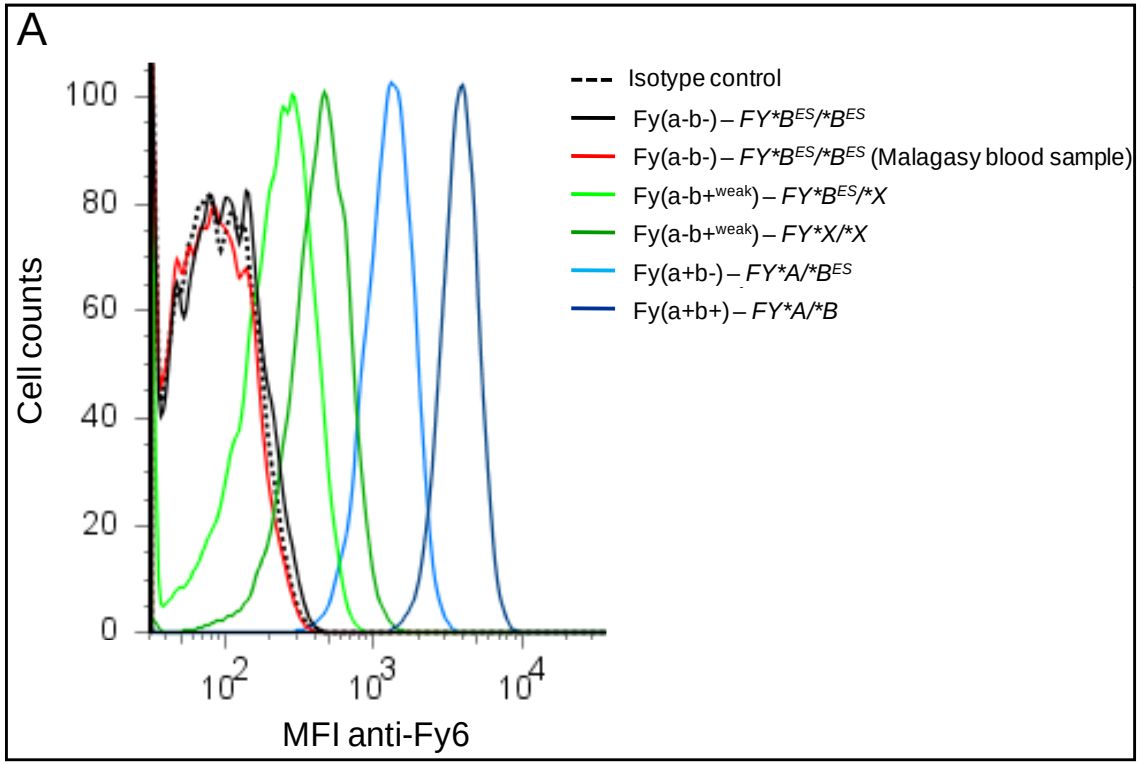
Evidence for intraerythrocytic development :asexual and sexual stages

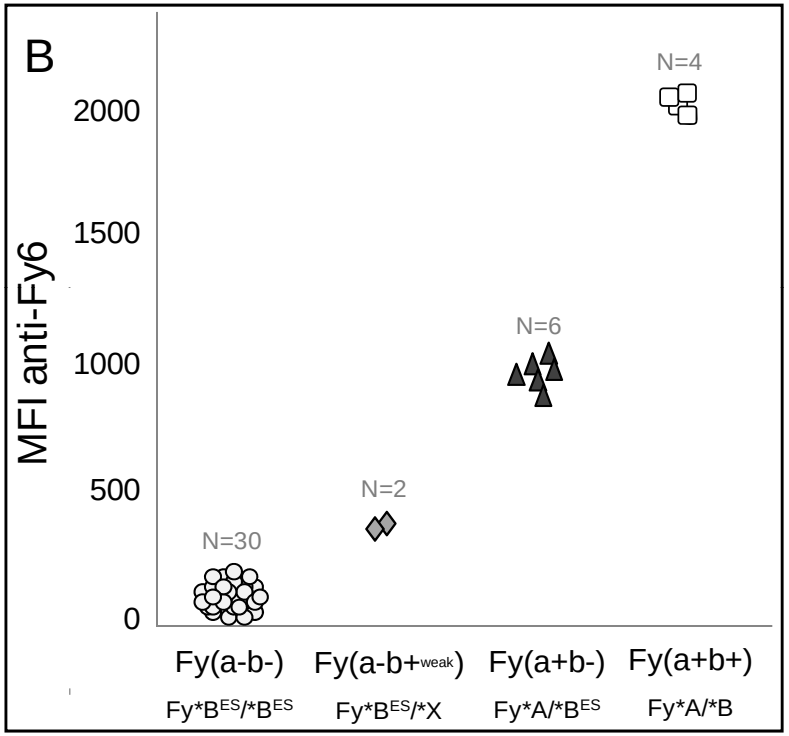
- 42 *P. vivax* infections (8.8%) in asymptomatic children genotyped Duffy negative
- 9 cases of *P. vivax* clinical malaria in Duffy negative patients (5.9%)
- Evidence of intraerythrocytic infections
- **Genotype…….. Phenotype??**

Concordance between Duffy genotype and phenotype (serotyping)

✓ New collection of samples from 43 schoolchildren from Tsiroanomandidy

- serology (DIAMED® card): 100% concordant with genotyping results
- flow cytometry (anti-Fy6 antibody labeling)





Mean fluorescence intensities reflecting the Duffy antigen specific anti-Fy6 antibody

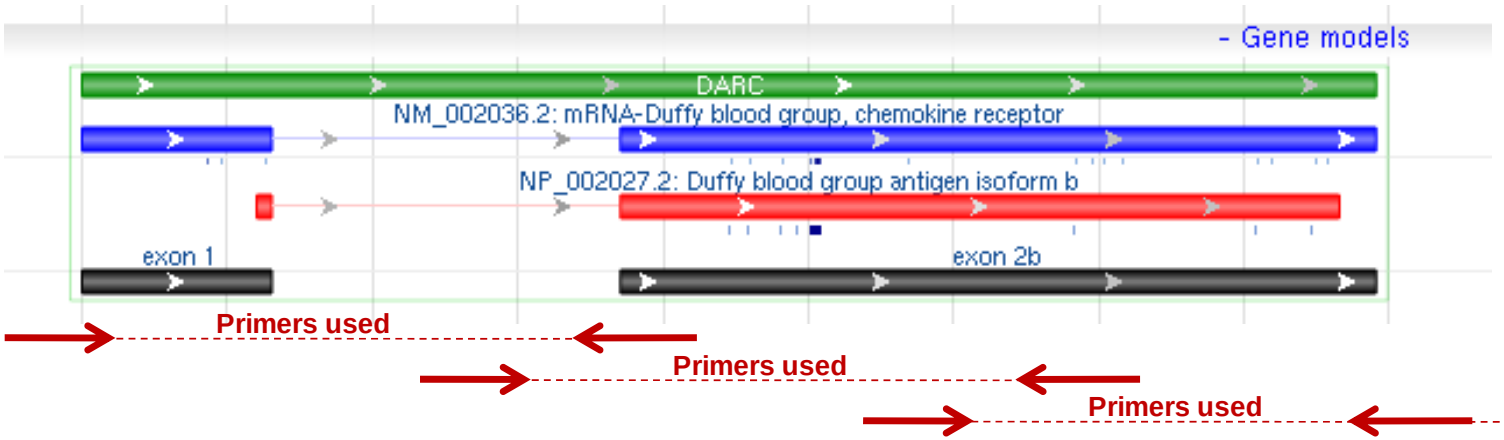
Extended Duffy gene sequencing

✓ Extended genotyping of Duffy blood group locus in 14 Duffy negative Malagasy patients :

1. single SNP (T > C) -33 in GATA-1 transcription factor binding site of the gene promoter that governs Duffy expression in erythroid cells

2. Intact, full length coding sequence

3. 100% concordance with 3 Duffy negative West African samples.



Genetic diversity of *P. vivax* strains infecting Duffy-negative Malagasies

Multilocus genotyping

1. Circumsporozoite protein (PvCSP)
   VK210, n=6; VK247, n=1; VK210 and VK247, n=9.

2. Microsatellites : 5 loci

Heterogeneity for individual loci was > 75% (No alleles/locus = 6-13)

>>> Multi-lineage background ; Duffy-neg =Duffy-pos

⇨ **multiple extant P. vivax strains carry the capacity to infect Duffy-negative erythrocytes**

Diversity of *P. vivax* Duffy Binding Protein

Duffy Binding domain Sequenced (N=243)

21 Alleles observed

Skewed distribution,

✓**8** alleles in Duffy negatives (n=27),

✓**21** alleles in Duffy positives (n=216)

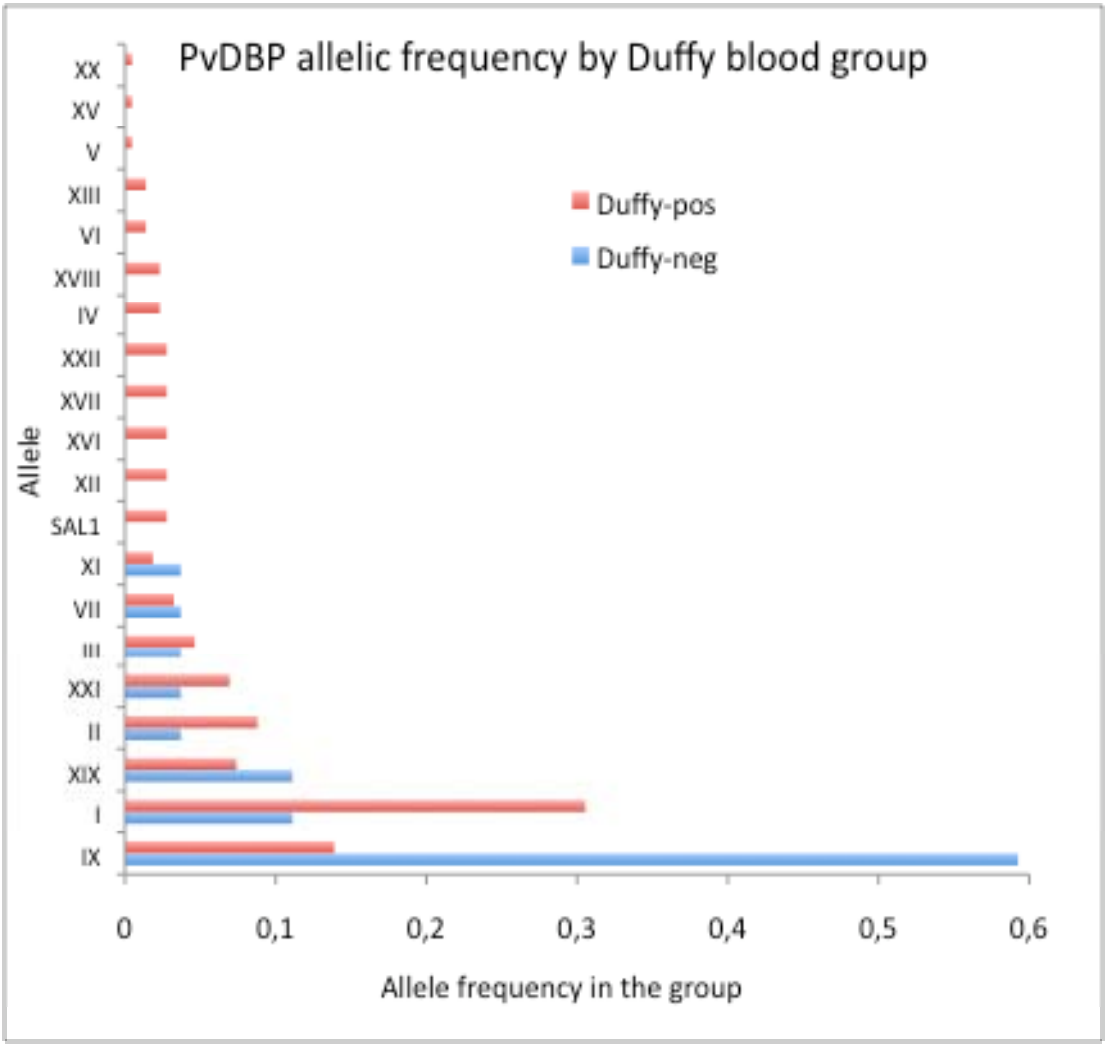
(Chi2, p<0.01).

In Tsiroanomandidy :

2 alleles in Duffy negatives (N=13)

8 alleles in Duffy positives (N=16)

(Chi2, p=0.007):

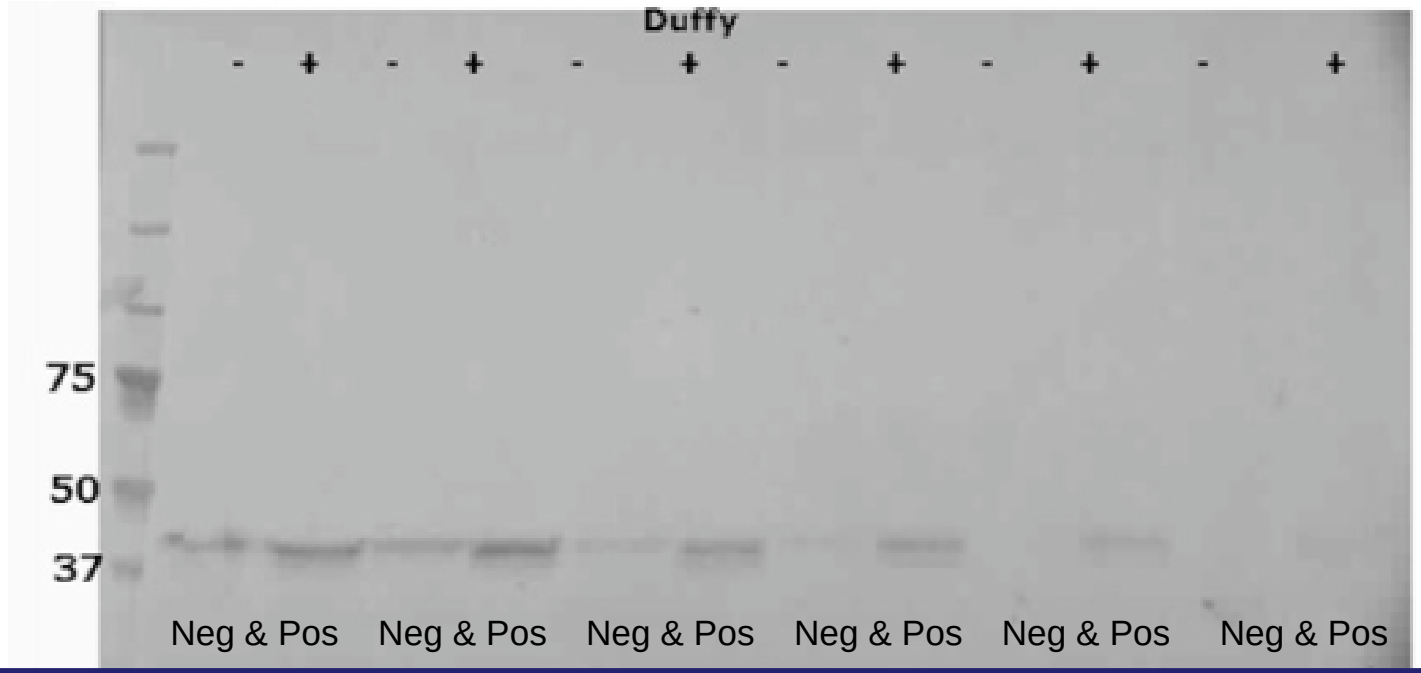


Allele IX much higher frequency in Duffy-negatives (P<10-6)

PvDBPallele IX binds Duffy-negative RBC

PvDBP allele IX: Baculovirus expression :
N glycosylation sites mutated
C-term His tag

(5x10^7 RBC/assay)

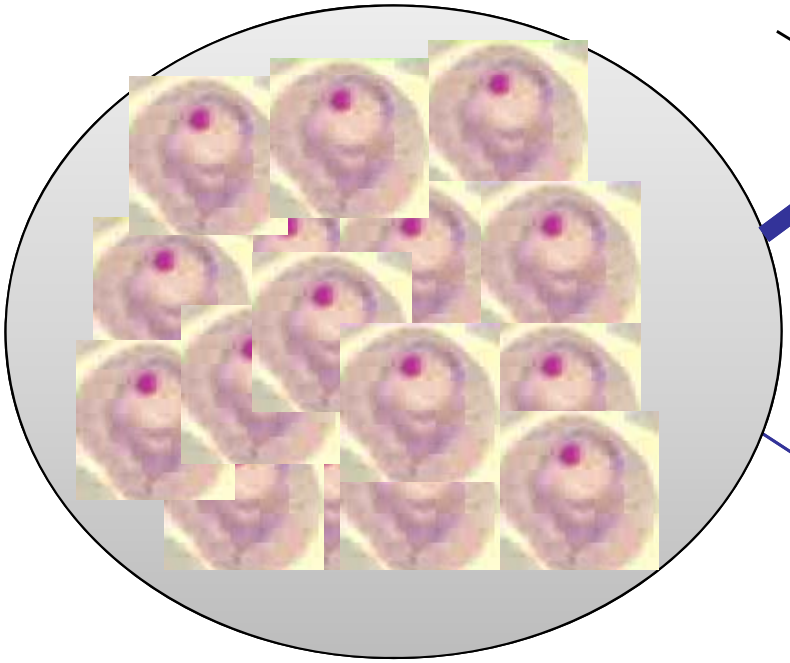


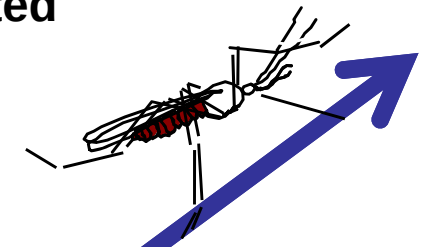
PvDBP (µg)	5	2.5	1	0.5	0.25	0.125

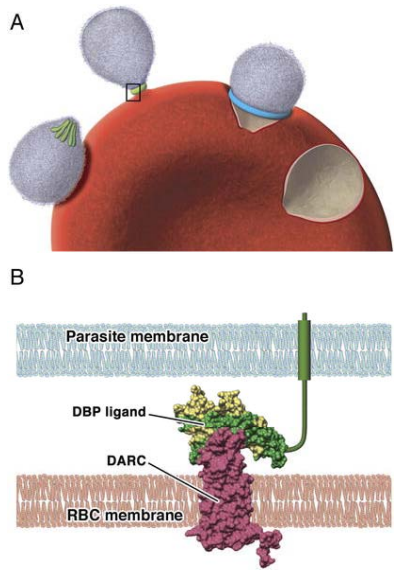
with reduced efficacy of binding compared to Duffy-pos RBC

Hypothesis

reservoir of Duffy positive infected carriers

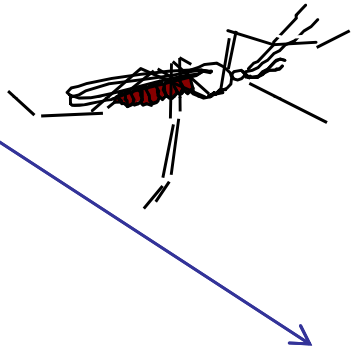


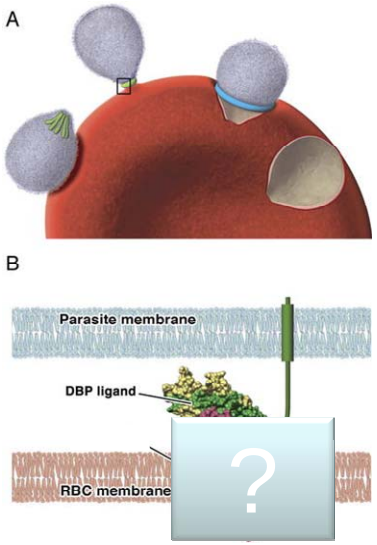




Duffy positive RBC

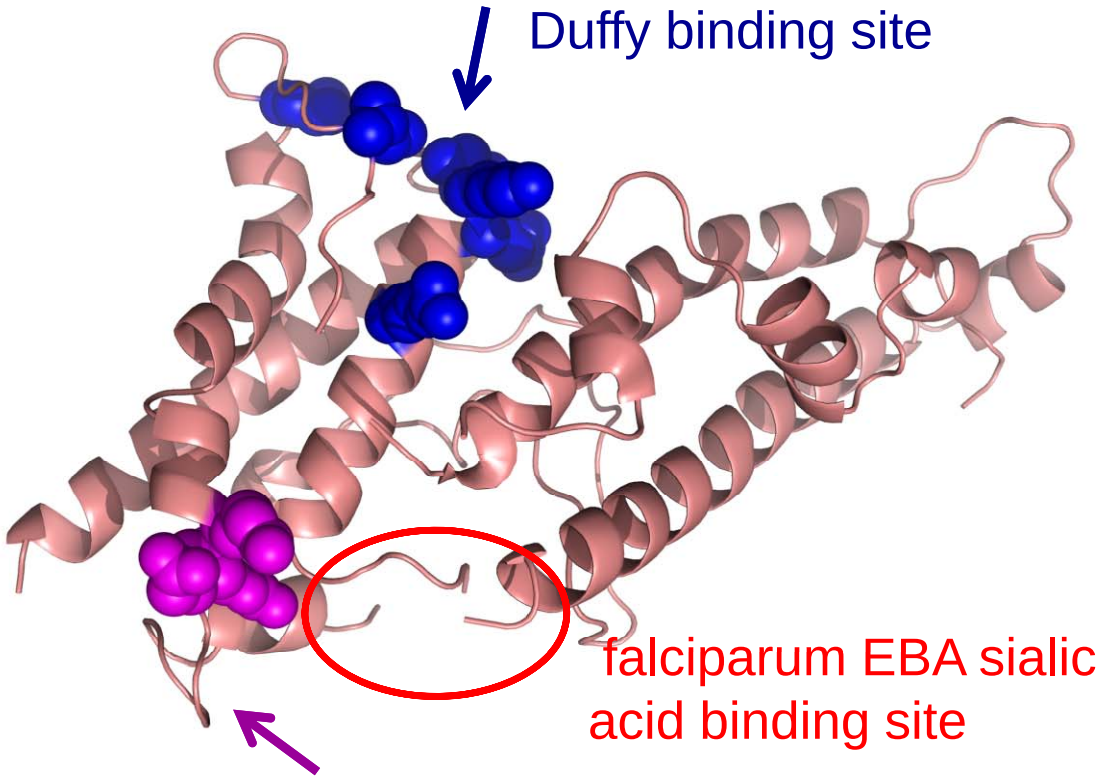
Invasion of through DARC pathway





Duffy negative RBC

Alternative invasion pathway (less efficient) but possibly involving mutant PvDBP.



✓ Surface exposed contact residues required for **recognition of DARC** on human erythrocytes are **conserved** in all *P. vivax* samples.

Dual functionality?

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