Evolution in action: lessons from antimalarial resistance

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ORIGINAL ARTICLE

Artemisinin Resistance in *Plasmodium falciparum* Malaria

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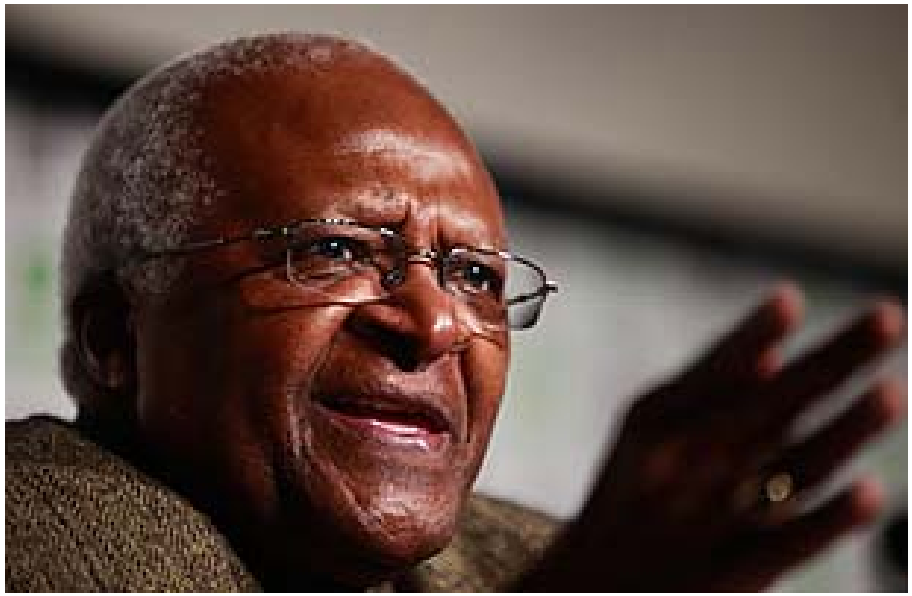
Chloroquine resistance in *Plasmodium vivax*

Molecular understanding

- Hundreds of pathogen, host and vector genomes sequenced

- Increasing individual genomes ‘resequenced’



Plasmodium falciparum genome

- 23 million bases in nuclear genome
- 14 chromosomes
- >5000 genes

- Non-globular domains within proteins

“Non-globular” domains

Proteins or domains that do not assume compact, folded structures

6 NOVEMBER 1998 VOL. 282 SCIENCE

Chromosome 2 Sequence of the Human Malaria Parasite *Plasmodium falciparum*

Malcolm J. Gardner, Hervé Tettelin, Daniel J. Carucci, Leda M. Cummings, L. Aravind, Eugene V. Koonin, Shamira Shallom, Tanya Mason, Kelly Yu, Claire Fujii, James Pedersen, Kun Shen, Jianping Jing, Christopher Aston, Zhongwu Lai, David C. Schwartz, Mihaela Pertea, Steven Salzberg, Lisim Zhou,* Granger G. Sutton,† Rebecca Clayton, Owen White, Hamilton O. Smith,† Claire M. Fraser, Mark D. Adams,† J. Craig Venter,† Stephan L. Hoffman‡

- 155/209 proteins had large **nonglobular** domains
  - expressed
- '… the *Plasmodium* genome sequence should provide broader biological insights, particularly in regard to … the preponderance of the predicted **nonglobular** domains in plasmodial proteins.'

PFE0195w amino acids 1-500

MARFCKSDKLLINHRELKYICLFYIILLFCFFLWCIFKKYHENRINRKHKR

KGSDSSHKNEKTEFFNYIYESYNDNDDVIIRQEGYKNSLCGFLLKNISIVL

YLLTHFIIILLIGNEYCIKENGEILWNDRAFVFFIFLLLCFIITYGILTVR

KHMHSFFIKPSLLKDSDYVLVYTKNEDYTNSYKNIFKESYVYITNVFIKWN

KKIYKYSCKYIKLLHYYQMNAKSFFFFISTDKKKKKNDYIKNSYHDDDLDD

DDIQNKDNNNYYYSNIYKKNSYYNNSFHKKSISNQYSNKRLSRNSLYTKKV

LRDQENNLGYYDQDQNEINKMNSIDHIYNIDSLNVIDQGNDKNYDNEEKEI

NKKYPFNRNDEYGDEYGDEYGDEYGNKYGNKYGNKYDDKYGNKYDDKYDDK

YDDKYDDKYDDKYDDKYDDKYDDKYDDKYDDKYDDNIVWGSKRFSNKKKKKGKKK

RKNEMVTSAQTNREYIENNLKVHKVKVRINEKNVRYFFFRS…

Pf non-globular domains 1998-2010

No function documented
- Deletion generally tolerated
- Missing sections in crystals
- Prone to SNPs and indels

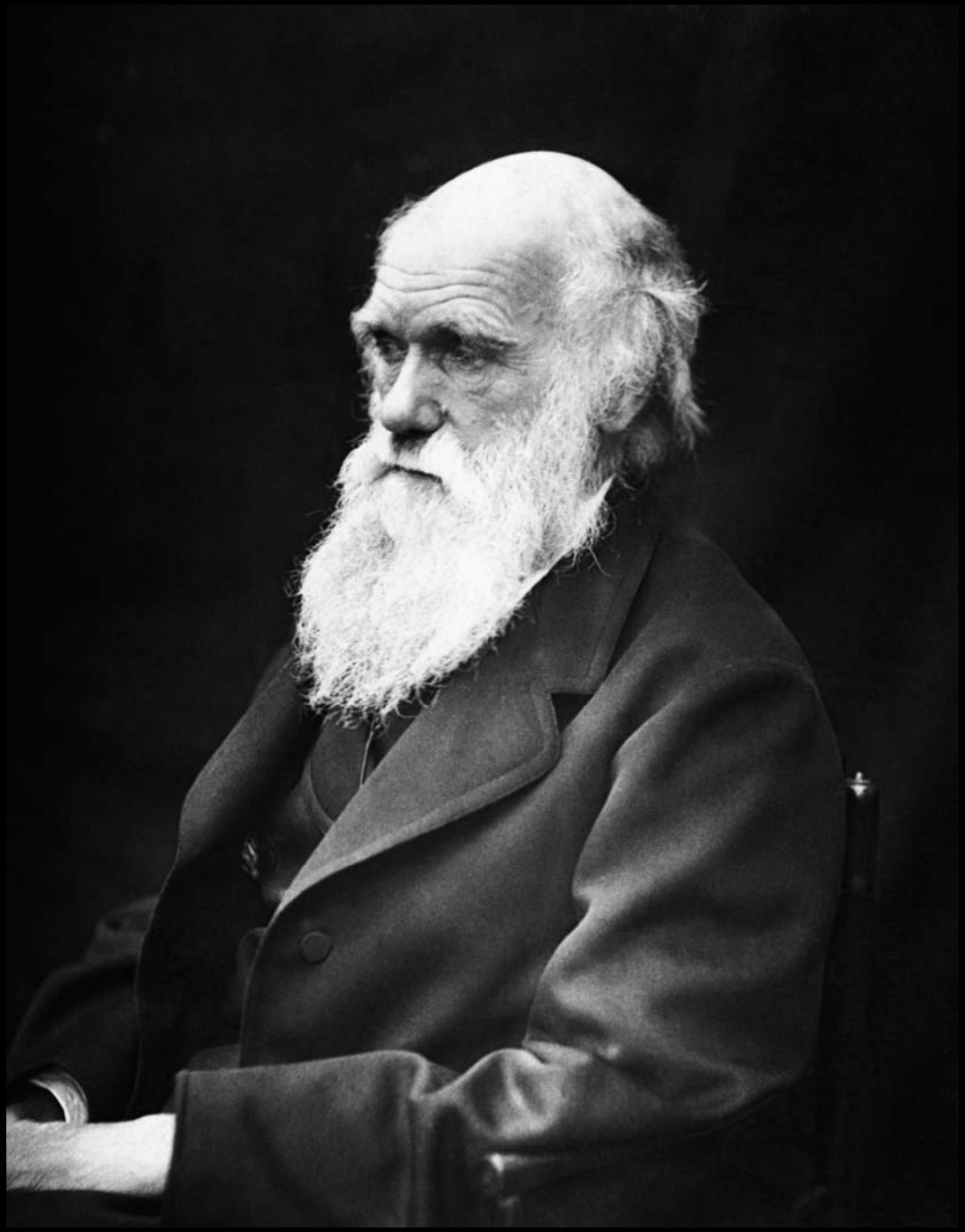
‘Neutral’

v

‘Adaptive’

SNPs and indels increasingly implicated in drug-resistance phenotypes
- artemisinins
- quinine
- doxycycline


The fog of information can drive out knowledge
Daniel J. Boorstin



Neutral evolution

"Variations neither useful nor injurious would not be affected by natural selection, and would be left a fluctuating element”

On the Origin of Species

1859

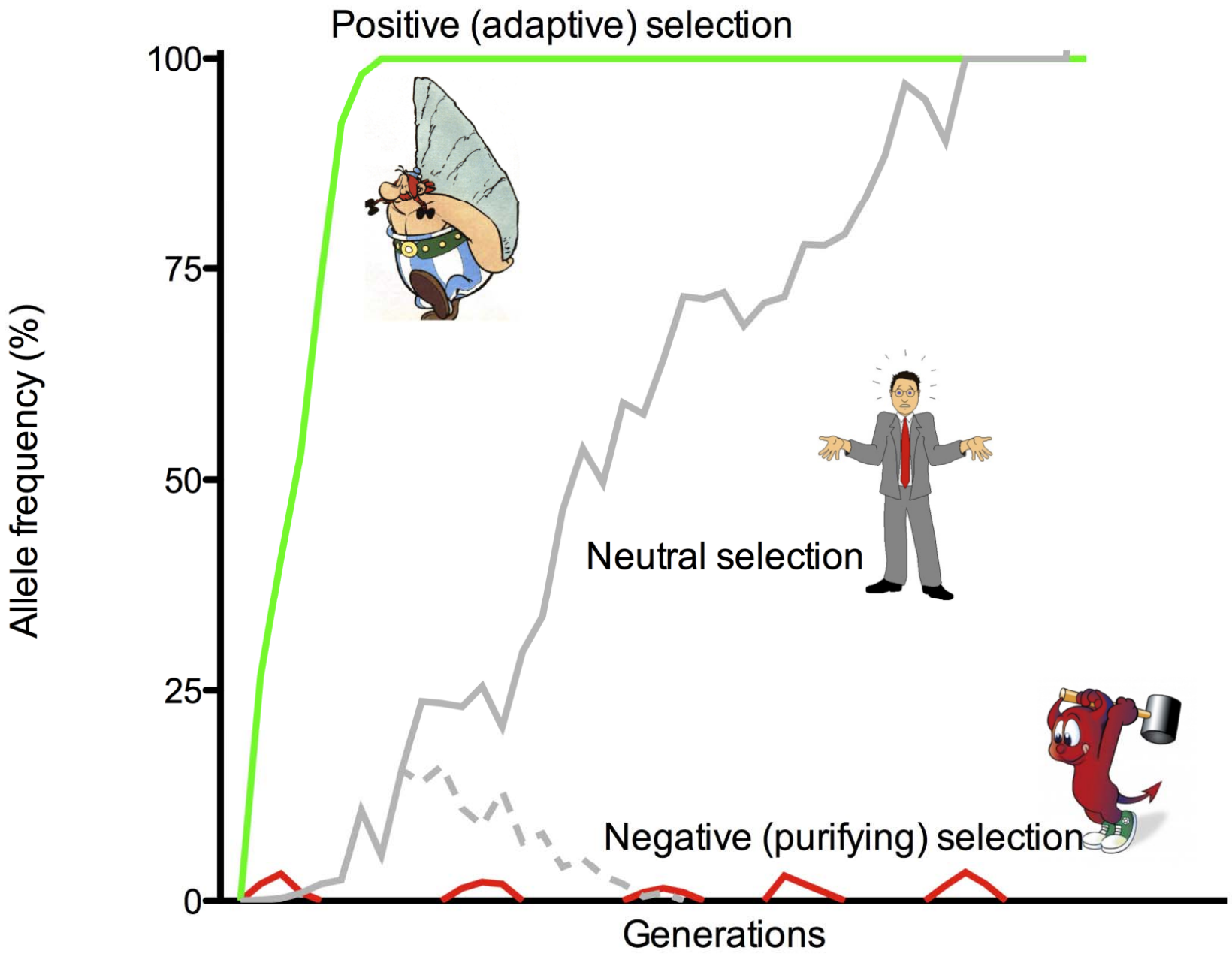
Neutral theory develops

- Kimura 1967
- King and Jukes

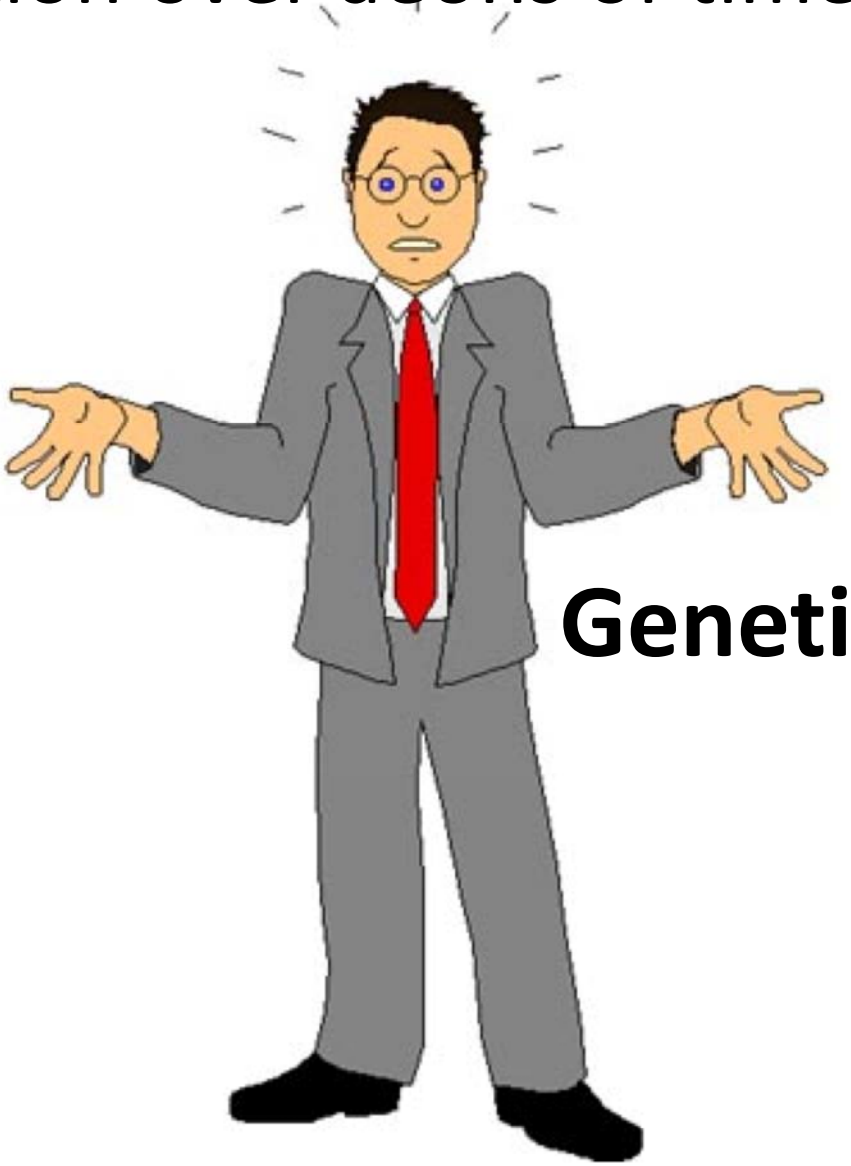
- At the molecular level, evolution is mostly neutral
  - e.g. synonymous mutations
  - non-synonymous mutations in haemoglobin

- Generally accepted
  - ? quantitative contribution to molecular evolution

3 ‘directions’ of selection



Kimura:
Molecular evolution over aeons of time



Genetic drift

Adaptive mutation

Adaptive change in natural world

? Neutral change in natural world

Loss of function

Cave salamander

Ostrich

Thai angel cave fish

Kakapo

What causes loss of function?

- Positive (adaptive)
  - Eyes are costly to make and can get infected
  - Wings are costly to grow and use up a lot of energy

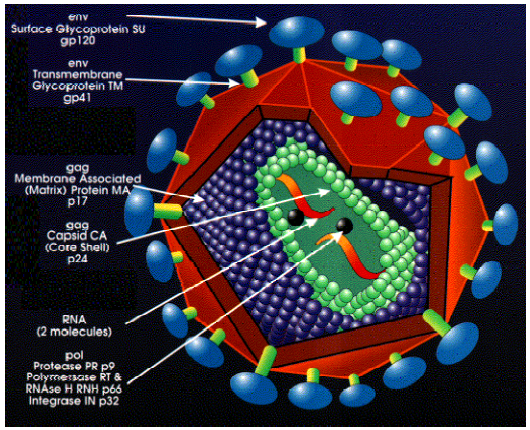
Mutations constantly bombard genome

Neutral perspective

- Genotype
  - Gene is freed from selective forces
- Under mutational bombardment
  - Non-synonymous polymorphism as common as synonymous
  - Insertion of stop codons (pseudogene)
- **Phenotype**
  - **Eyes and wings are lost because negative selection is not maintained**

Exploring neutral theory in infectious diseases

First drugs against HIV

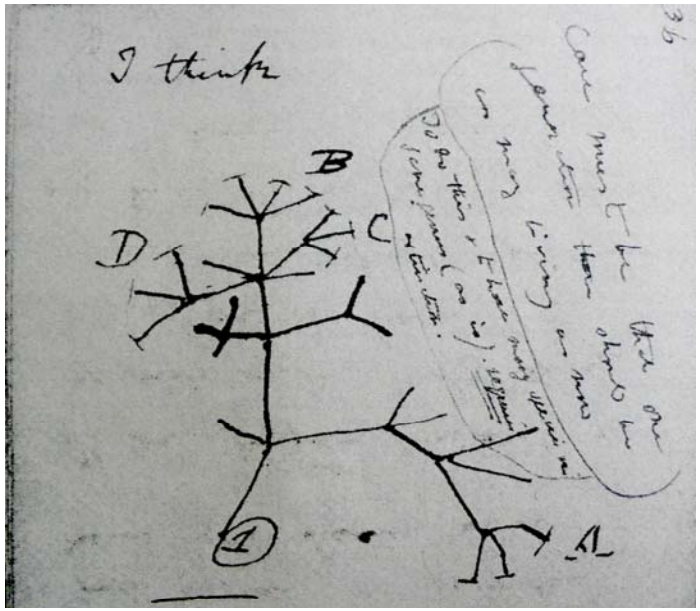


- Inhibit reverse transcriptase
- Nucleoside reverse transcriptase inhibitors (NRTIs)

- Resistance : predictions from neutral theory
  - Conservation of amino acid residues involved in drug-binding site
  - Resistance emerges via mutation at conserved residues
  - Fitness cost

How do we test this?

- Use evolutionary comparison
- Compare to orthologous sequences from other species
  - HIV-2, etc.



Lentivirus reverse transcriptases

```
HIV1.prot          1 -PISPIDTVPVTLKPGMDGPKVKQWPLTEEKIKALTEICKEMEKEGKISKIGPENPYNTP  59
Equine IA.prot     1 -------------------------LTTAHYNRLKYRGRKYQGTGIIGVGGNVETFSTP  34
FIV.prot           1 -------------------------LSNEKIEALTDIVERLESEGKVKRADPNNPWNTP  34
SIV.prot           1 -PIAKVEPVKVTLKPGKDGPKLRQWPLSKEKIVALREICEKMEKDGQLEEAPPTNPYNTP  59
HIV2.prot          1 MAVAKVEPIKIMLKPGKDGPKLRQWPLTKEKIEALKEICEKMEKEGQLEEAPPTNPYNTP  60
                                          *.  .   *       .  * .          **

HIV1.prot         60 VFAIKKKDSTKWRKLVDFRELNKRTQDFWEVQLGIPHPAGLKKKKSVTVLDVGDAYFSVP 119
Equine IA.prot    35 VTIKKKGRHIKTRMLVAD---------IPVTILGRDILQDLGAKLVLAQLSKEIKFRKIE  85
FIV.prot          35 VFAIKKKSG-KWRMLIDFRVLNKLTDKGAEVQLGLPHPAGLQMKKQVTVLDIGDAYFTIP  93
SIV.prot          60 TFAIKKKDKNKWRMLIDFRELNKVTQDFTEVQLGIPHPAGLAKRKRITVLDVGDAYFSIP 119
HIV2.prot         61 TFAIKKKDKNKWRMLIDFRELNKVTQDFTEIQLGIPHPAGLAKKRRITVLDVGDAYFSIP 120
                        **    * * *.                 **      *  .  .. *     .  .

HIV1.prot        120 LDESFRKYTAFTIPSMNNETPGIRYQYNVLPQGWKGSPAIFQSSMTKILEPFRIKNPEMV 179
Equine IA.prot    86 LKEGTMGPKIPQWPLTKEKLEGAKEIVQRLLSEGKISEASDNNPYNSPIFVIKKKSGKWR 145
FIV.prot          94 LDPDYAPYTAFTLPRKNNAGPGRRYVWCSLPQGWVLSPLIYQSTLDNILQPFIKQNPELD 153
SIV.prot         120 LDEEFRQYTAFTLPSVNNAEPGKRYIYKVLPQGWKGSPAIFQHTMRNVLEPFRKANPDVT 179
HIV2.prot        121 LHEDFRPYTAFTLPSVNNAEPGKRYIYKVLPQGWKGSPAIFQHTMRQVLEPFRKANKDVI  18
                      *           *       *.     *      *     .      .
```

Resistance to NRTIs determined by RT K65R

Drug resistance in highly conserved location

Fitness cost

Principle seems to apply

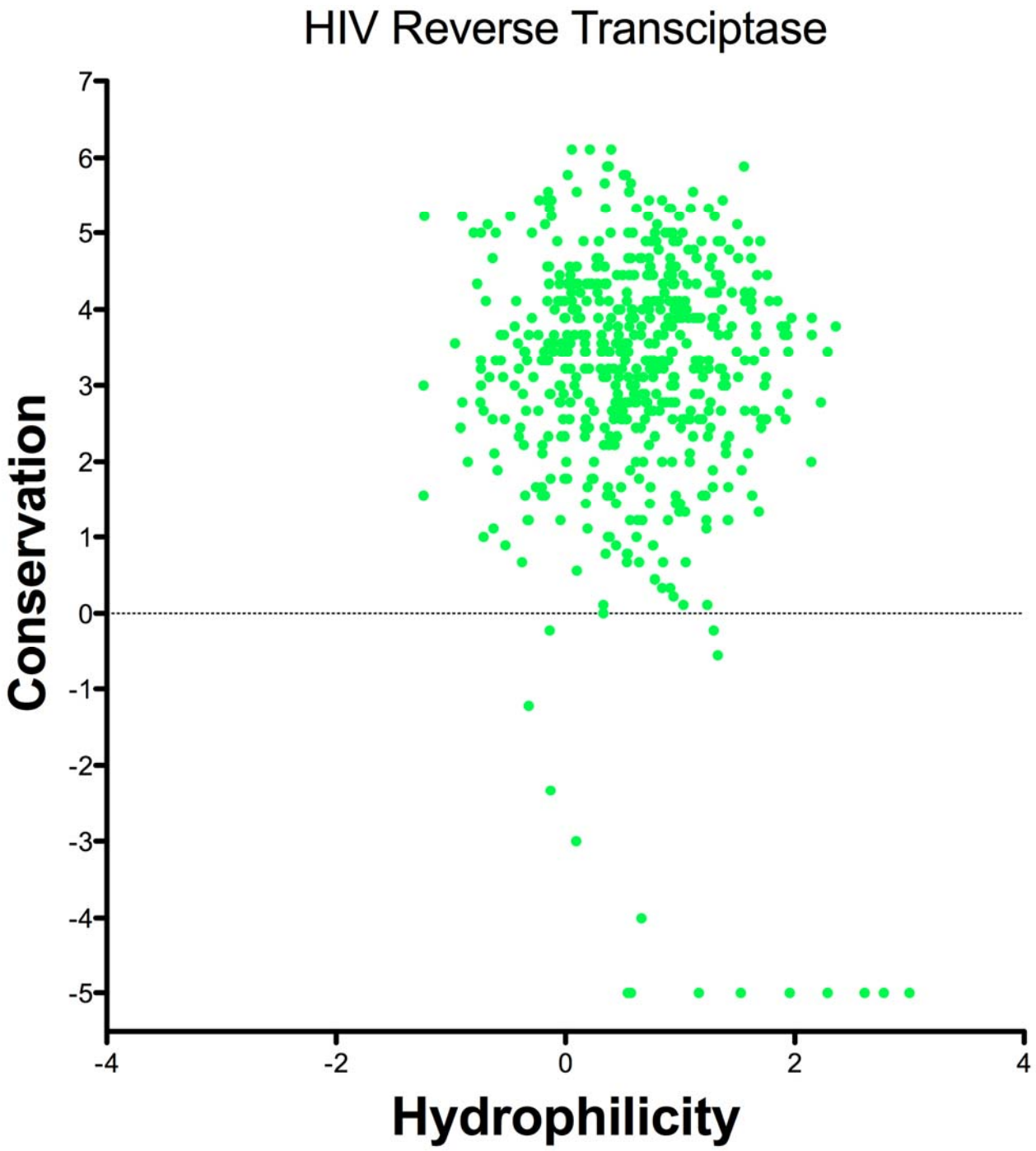
Non-globular regions in *P. falciparum*:

Methods to define non-globular regions

- (Informational complexity)

- **Evolution**
  - Other *Plasmodium* species genomes now available
  - *P. chabaudi* (rodent)
  - Conservation score across ortholog

- Note hydrophilicity to add structural component


HIV Reverse Transciptase
Conservation
Hydrophilicity
7
6
5
4
3
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-1
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-3
-4
-5
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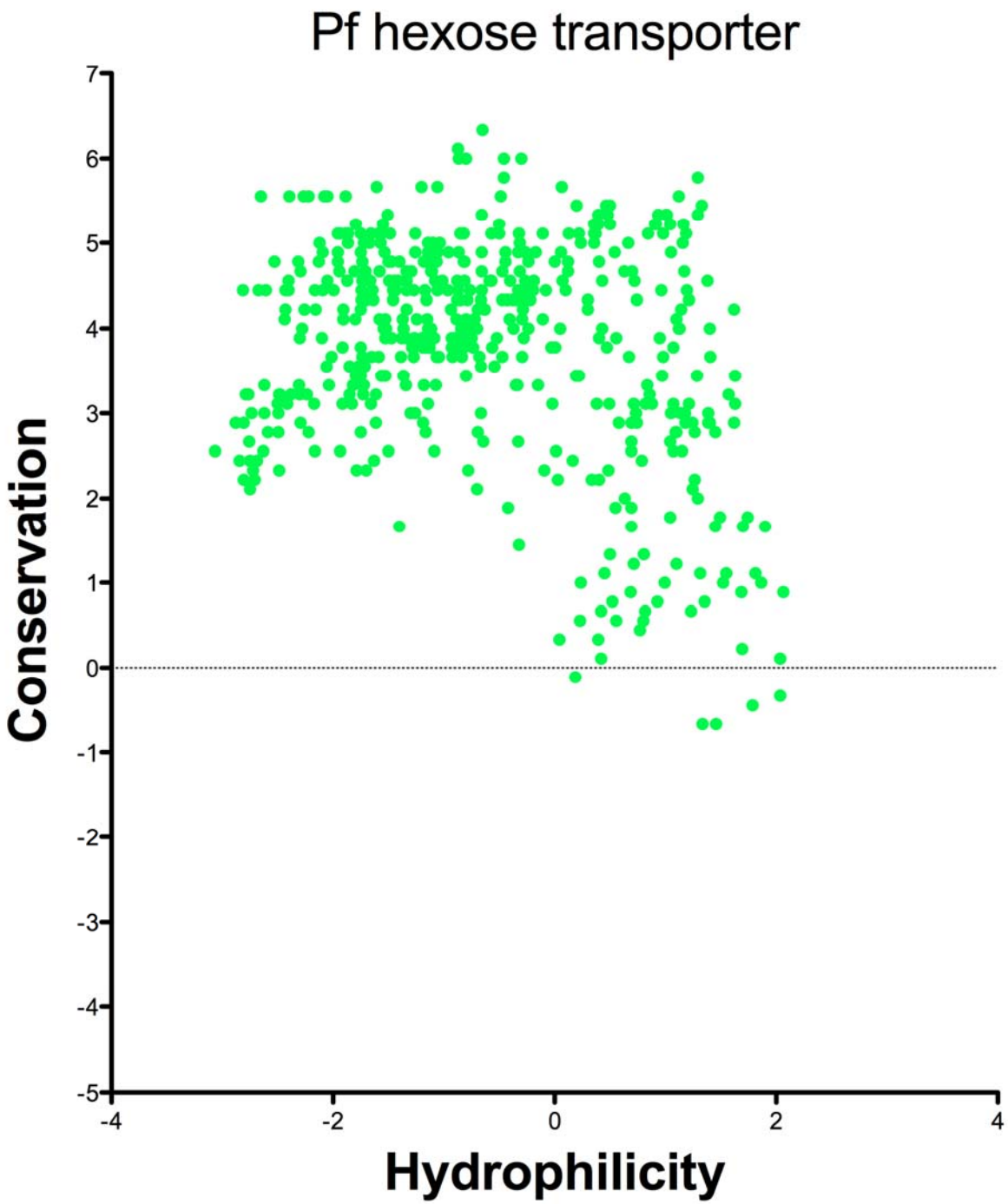
-2
0
2
4


Pf hexose transporter
Conservation
Hydrophilicity
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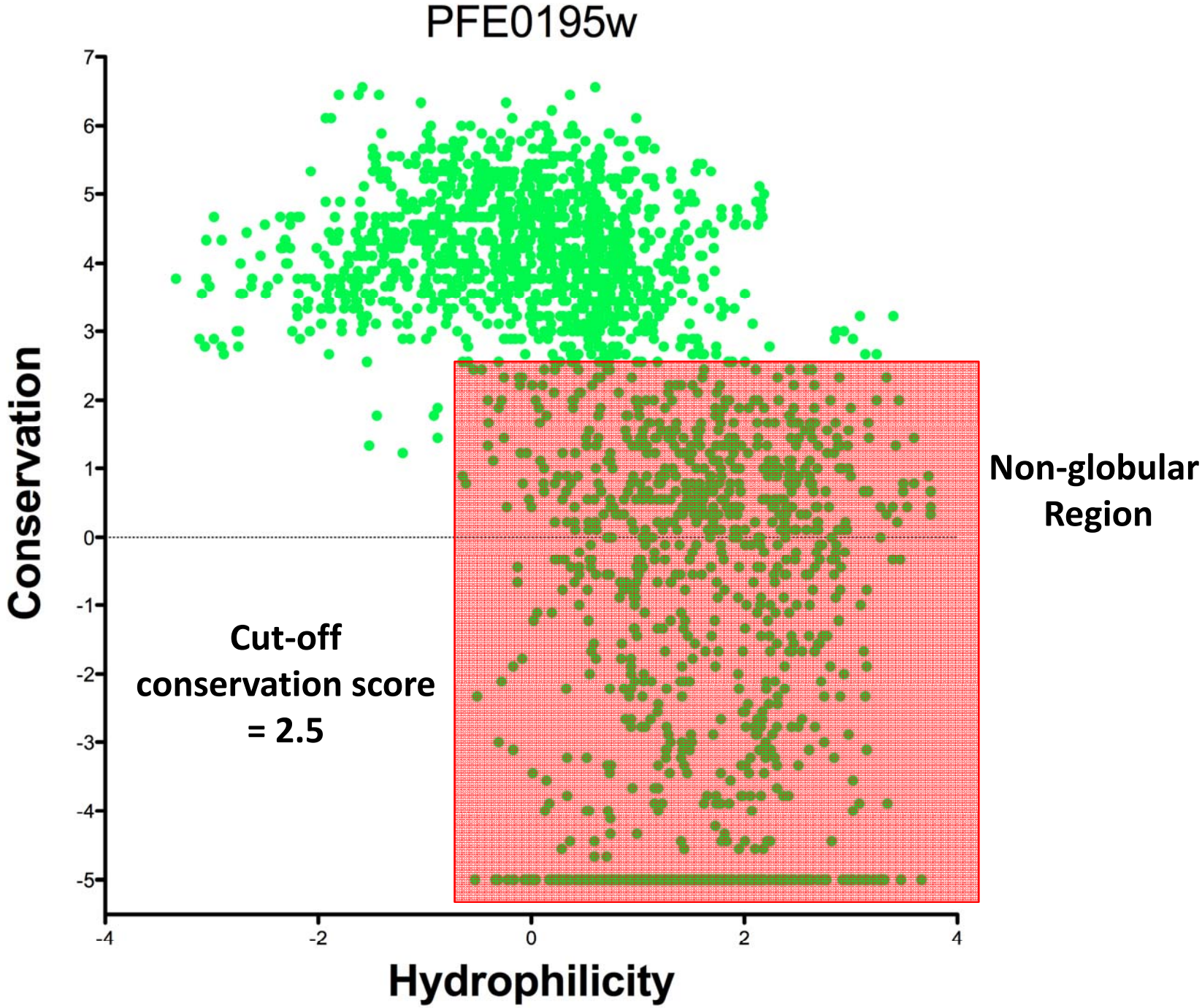
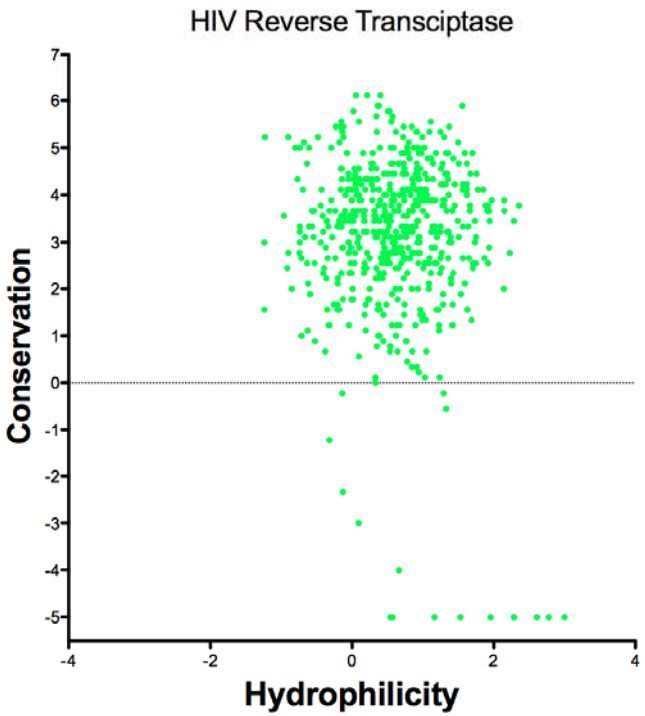

PFE0195w
Conservation
Hydrophilicity
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-4
-2
0
2
4
Cut-off
conservation score
= 2.5
Non-globular
Region


HIV Reverse Transciptase
Conservation
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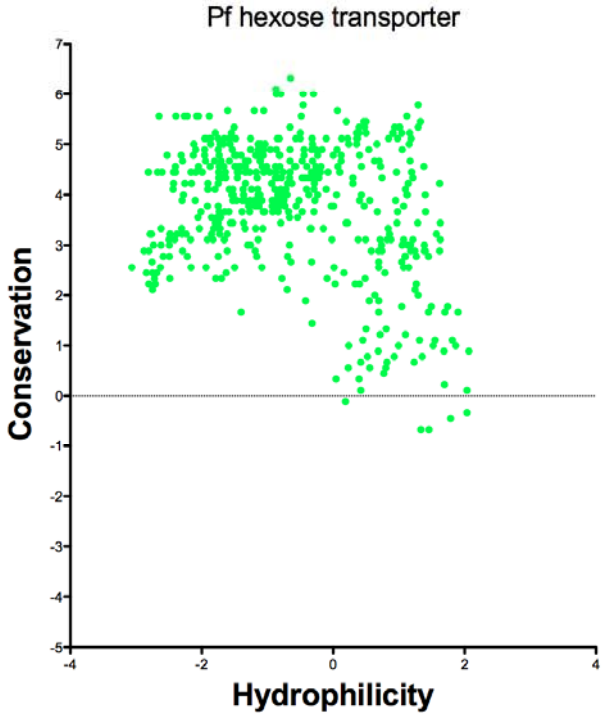
Pf hexose transporter
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Hydrophilicity
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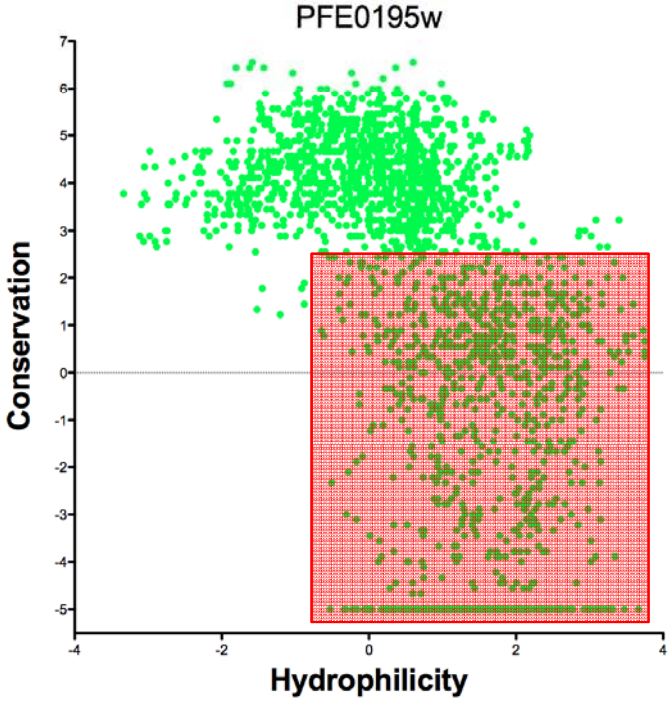
PFE0195w
Conservation
Hydrophilicity
7
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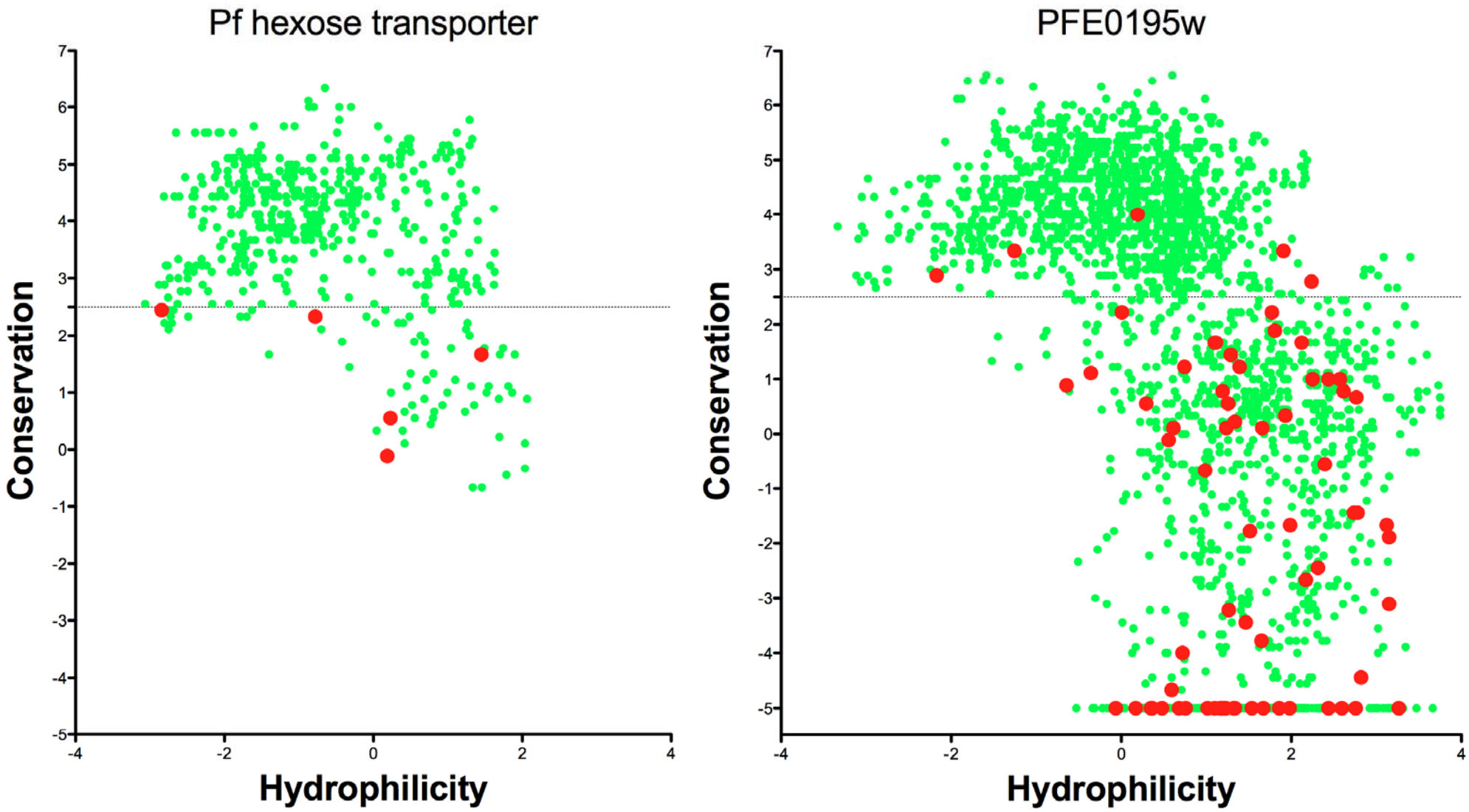
4

Examine non-synonymous fixed differences within clade

P. falciparum (human) vs.
P. reichenowi (chimpanzee)


Pf hexose transporter
PFE0195w
Conservation
Hydrophilicity
7
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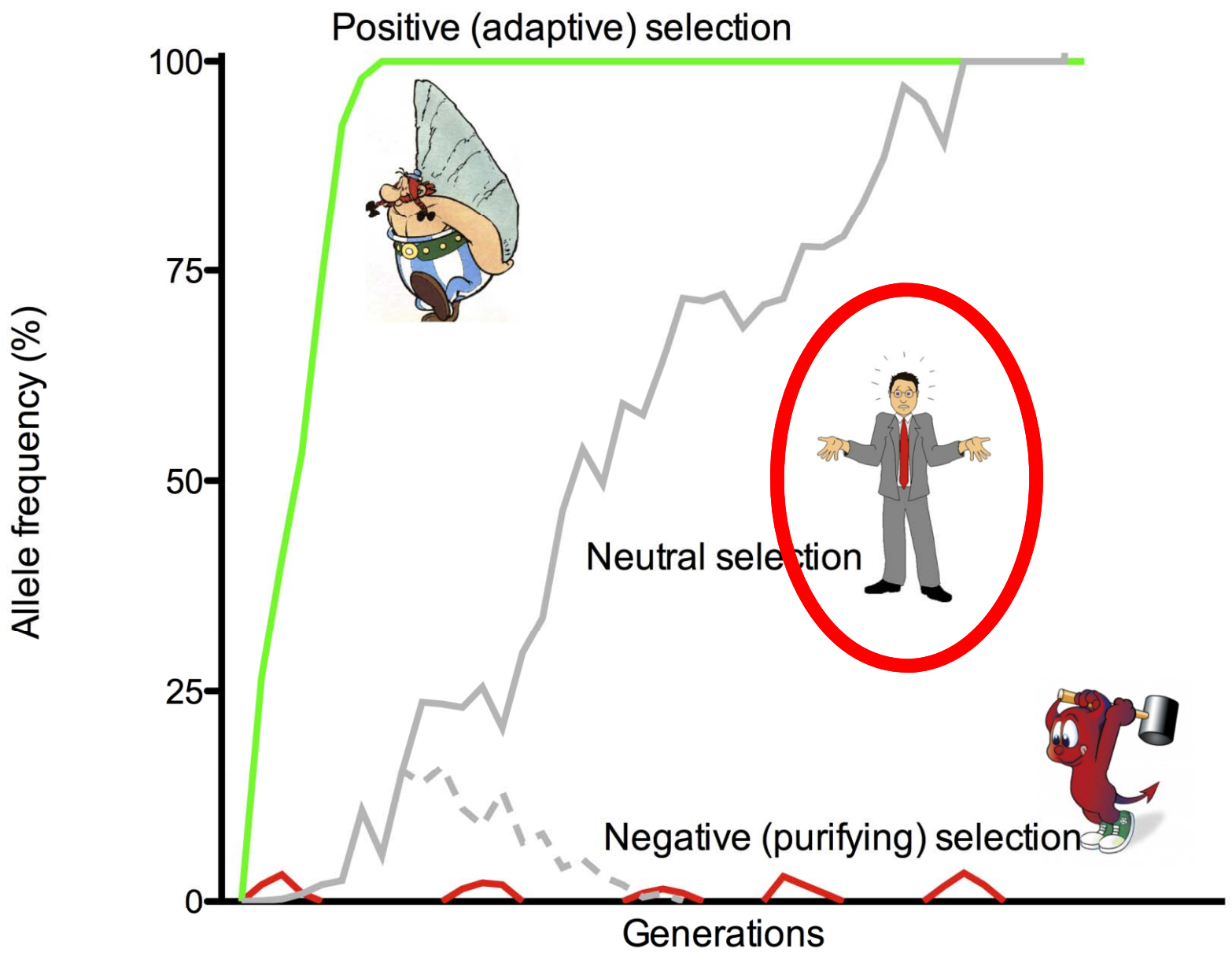
-1
-2
-3
-4
-5
-4
-2
0
2
4

Extension to 40 genes

- Fixed differences between *P. falciparum* and *P. reichenowi* follow neutral theory
  - Genetic drift
- Most mutations appear to be in non-globular regions
  - Non-synonymous mutation 20 times more common than in globular regions

Genetic drift appears most common form of mutation



Functional mutations

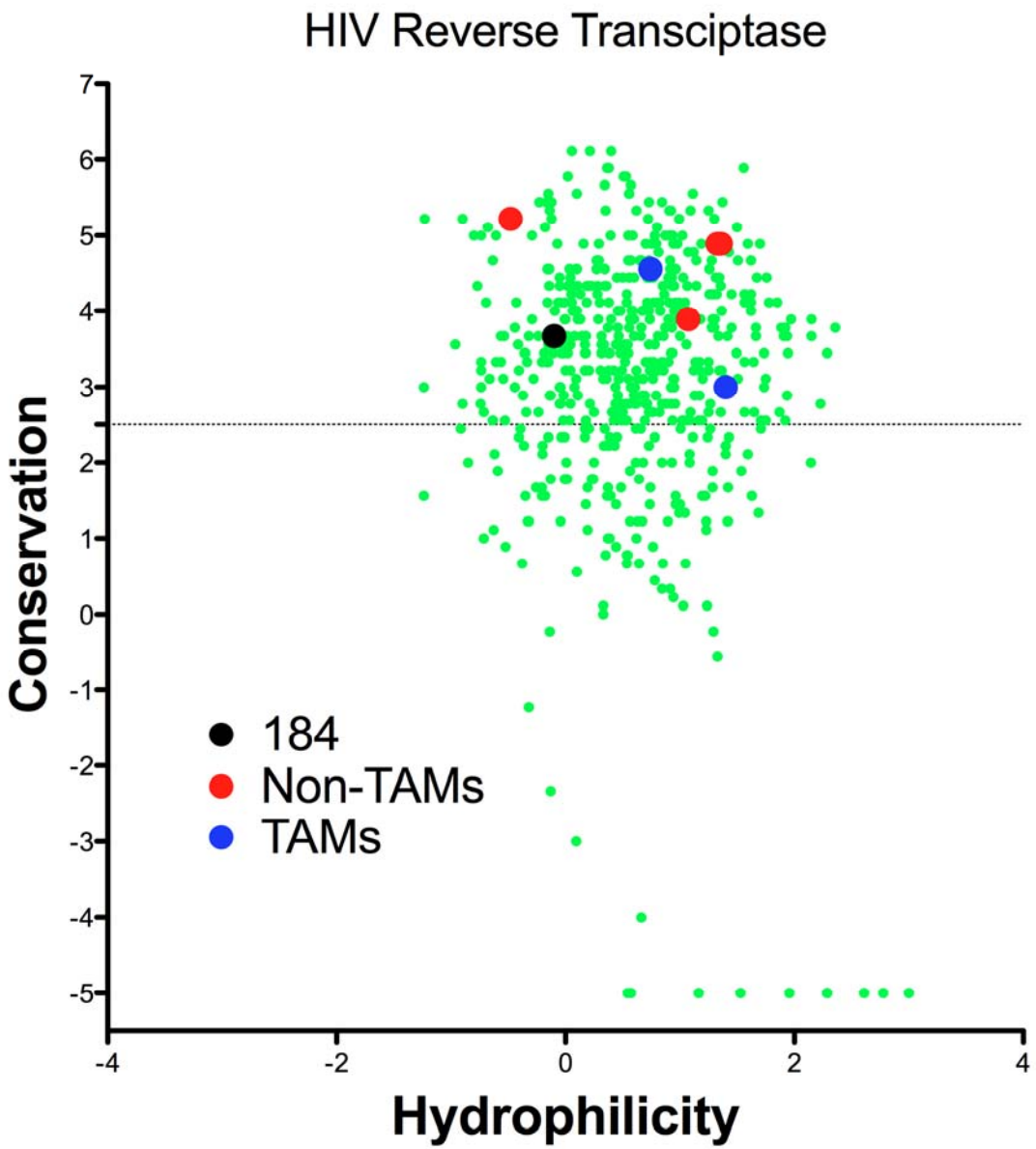
- Should show opposite pattern

Functional mutations in enzymes = **Drug-resistance mutations**

- Drug-resistance also most practical application

Test approach in HIV reverse transcriptase

NRTI mutations

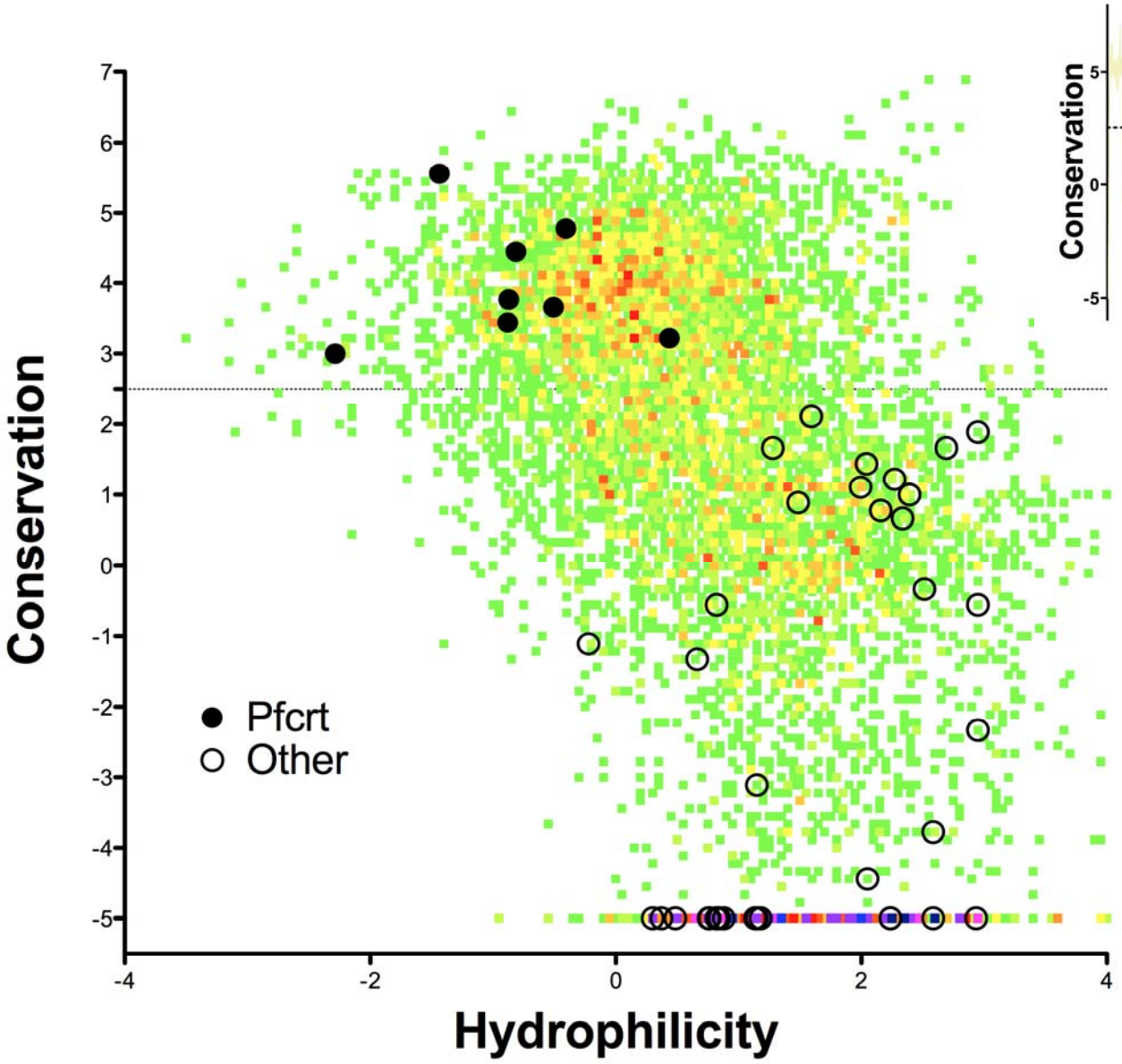


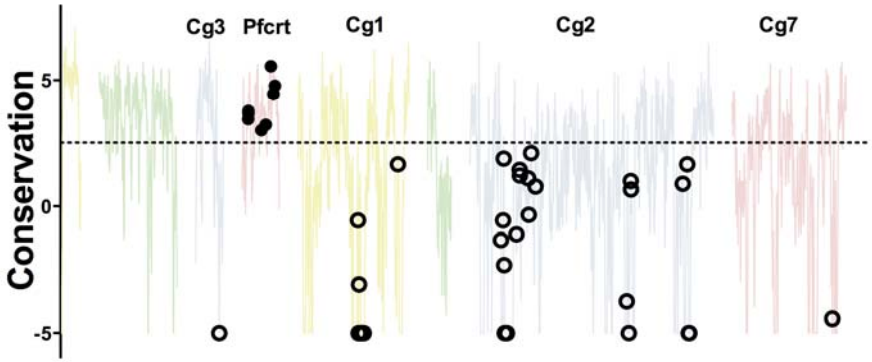
Apply to known antimalarial resistance loci

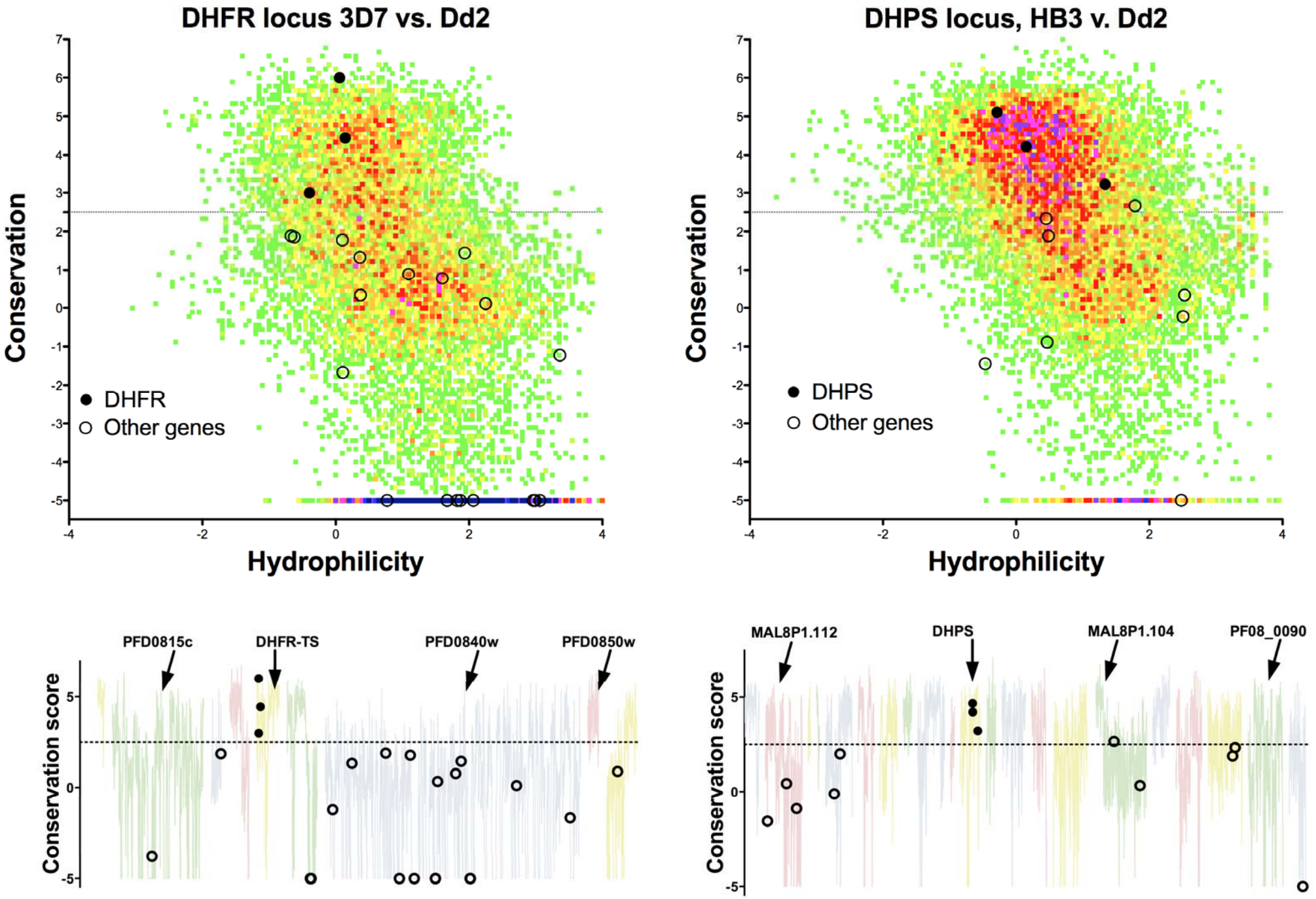
Compare **sensitive** and **resistant** strains

Chloroquine resistance locus

HB3 vs. Dd2® nSNPs






DHFR locus 3D7 vs. Dd2
Conservation
Hydrophilicity
DHFR
Other genes
DHPS locus, HB3 v. Dd2
Conservation
Hydrophilicity
DHPS
Other genes
PFD0815c
DHFR-TS
PFD0840w
PFD0850w
Conservation score
MAL8P1.112
DHPS
MAL8P1.104
PF08_0090
Conservation score

Performance

- Tested in other independent isolate pairs
- Sensitivity / specificity not straightforward because usually >1 mutation

- Accuracy appears ≥ 95%

Conclusion

- *Plasmodium* proteins are rich in non-globular domains
- Most polymorphism occurs within these (genetic drift)
  - Last place to look for functional mutations

- Conserved regions
- Pairwise non-synonymous mutations are very rare

- Known drug resistance mutations are conspicuous in their occurrence in highly conserved regions
- Such regions have experienced more mutation in decades of drug use than millions of years of natural selection

Thank you

Kate Gardner

Ipsita Sinha

Nick Day

Nick White

(MORU)

Leyla Bustamante

(Sanger, UK)