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Competition, cooperation and immune selection for plasmodium falciparum malaria

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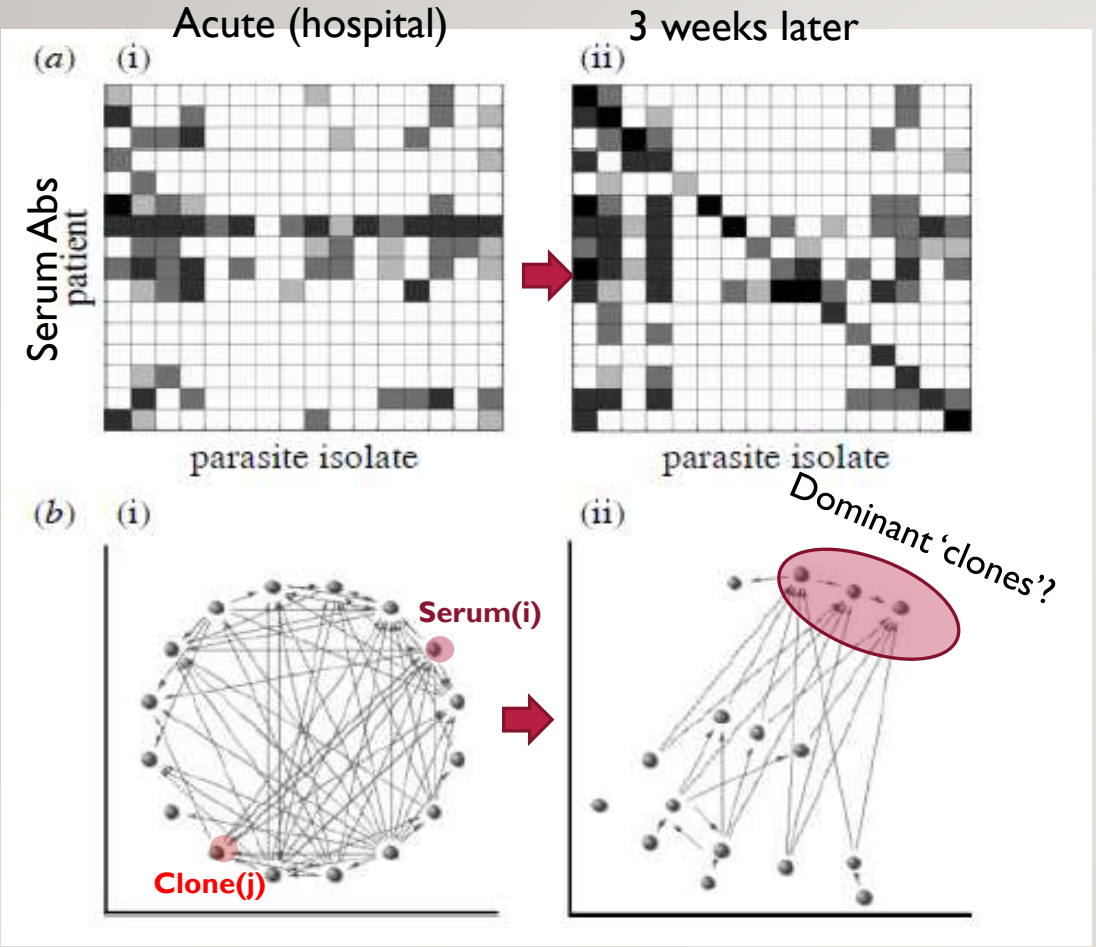
Parasite-immune interactions

- Driving forces of parasite evolution and selection:
  - Parasite-immune interactions + transmission environment
- Implications: vaccine design, prediction, control mitigation
- Examples:
  - Respiratory pathogens (Covid19, flu, RSV…): evolution, predictability ??
  - Effect of vaccination
  - Malaria (A. Reed 2008): "Can imperfect vaccine drive evolution of 'virulence'?

Evidence for clone interaction via cross-reactive serology



Disordered initial state

Organized 'dominance structure'

- Cross-reactive samples: parasite (clones) – serum (Ab)
- Transition: acute -> post-infection status

Dominance network structure

C. O. Buckee *et al.* *Malaria parasite population structure*

Proc. R. Soc. B (2009) **276**, 477–485
doi:10.1098/rspb.2008.1122

Modeling work (snapshot)

Evolution of virulence by imperfect ***vaccine***

- Mackinnon et al, Vaccine, 2008

Strain-selection theory: *immunity* shaping parasite population structure in host communities

- Gupta, S., et al. Science 280, (1998)
- Gupta, S. et al. Nat. Med. 2, 437–442 (1996)

Population-based

- Severins, M. et al, JRSI (2011)
- Buckee, C. O., et al, PNAS 108, (2011).

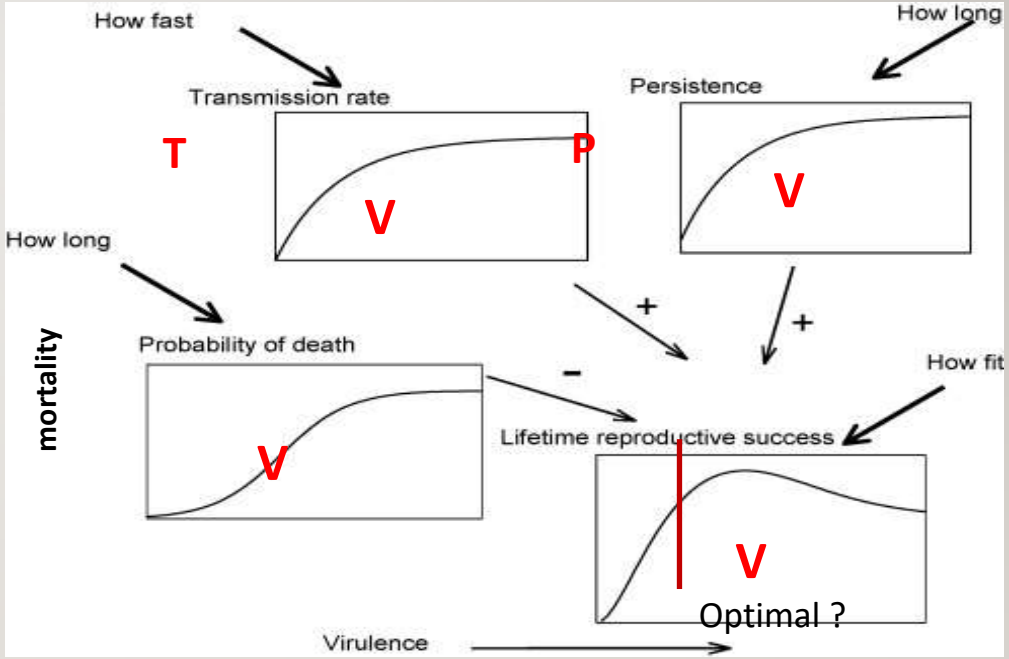
ABM

- He, Q. et al, Nature Com, (2018)

Key concepts

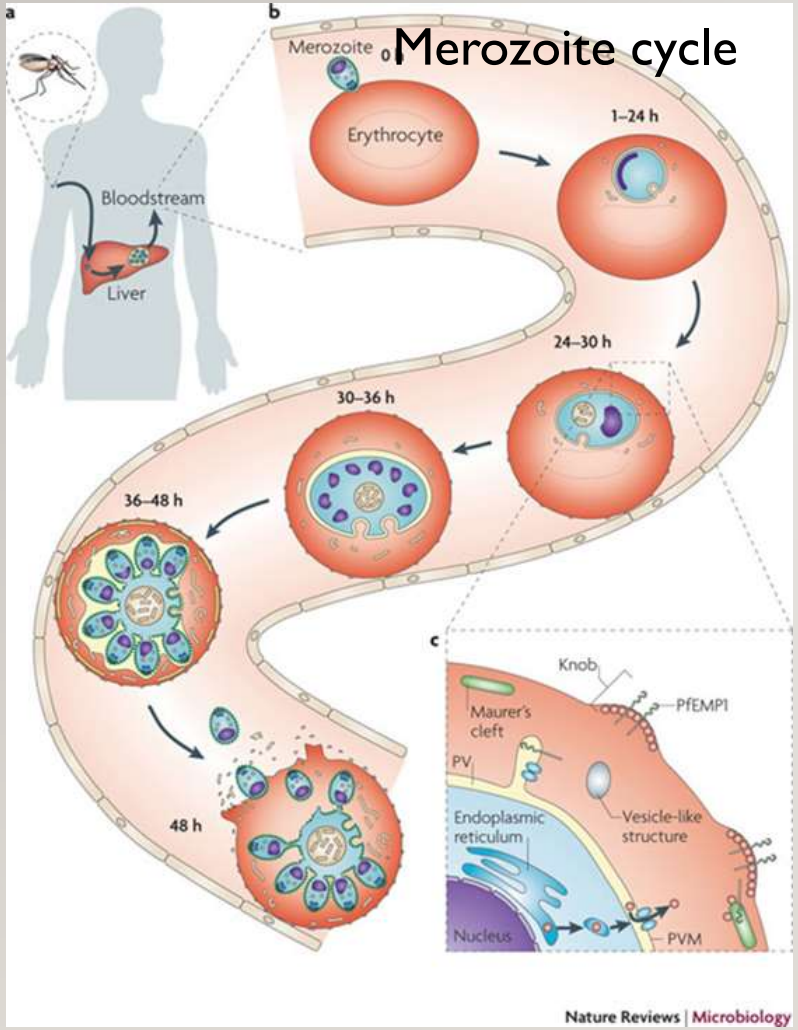
- P genetic makeup
- P-immune interaction, cross-reactivity CR
- Fitness traits: **V-T-P** (virulence-transmissibility-persistence)

Interplay between **V-T-P** and their trade-off can drive evolution of virulence

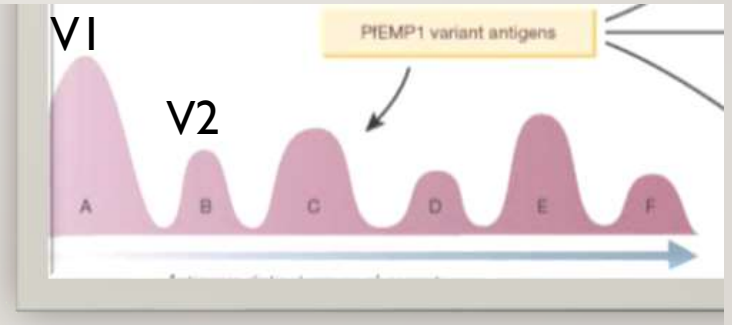


Can these conceptual ideas be tested with detailed models of host-parasite0-immune biology ?

Malaria biology: life-cycle, immune evasion



- **Human:** *merozoite* cycle (2 days) subject to *immune control*
- **Mosquito:** gametocyte uptake, mating and *gene crossover*
- ***Immune evasion*** *via* ***AV*** (antigenic variation)
- **iRBC** express Pf *variable surface antigens* (VSA) encoded by 50-60 ***var-genes***
- Each cycle has single expressed **Var**, can switch on the next cycle
- ***Vars*** stimulate specific Abs that clear clones with expressed Var
- Vascular sequestration and severe malaria due to Vars



Multiple P. waves arising from VSA expression

ABM modeling highlights

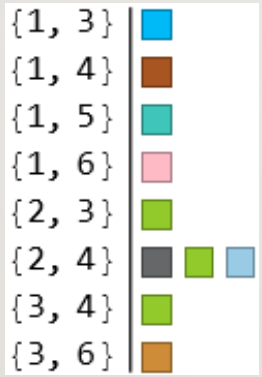
- Genetically structured Pf: clone = collection of Vars
- Host agent state
  - Target RBC, infected iRBC (w. expressed vars), gametocytes (all cell populations/[μL]):
  - Immunity
    - Innate: febrile threshold for cumulative iRBC-load
    - Adaptive: Var-specific Abs
  - Processes:
    - RBC: invasion/depletion/production
    - iRBC: merozoite replication, AV-switch, gametocyte production
    - Immunity: stimulation/clearing of (expressed) clones, immune loss
- Transmission: mosquito uptake/ inoculation (EIR), crossover

Genetically structured parasite (Pf)

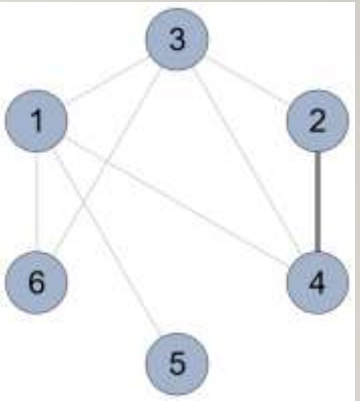
- Natural Var-gene repertoire:

- 6 clones:

Shared Vars



CR immune network for 6 clones

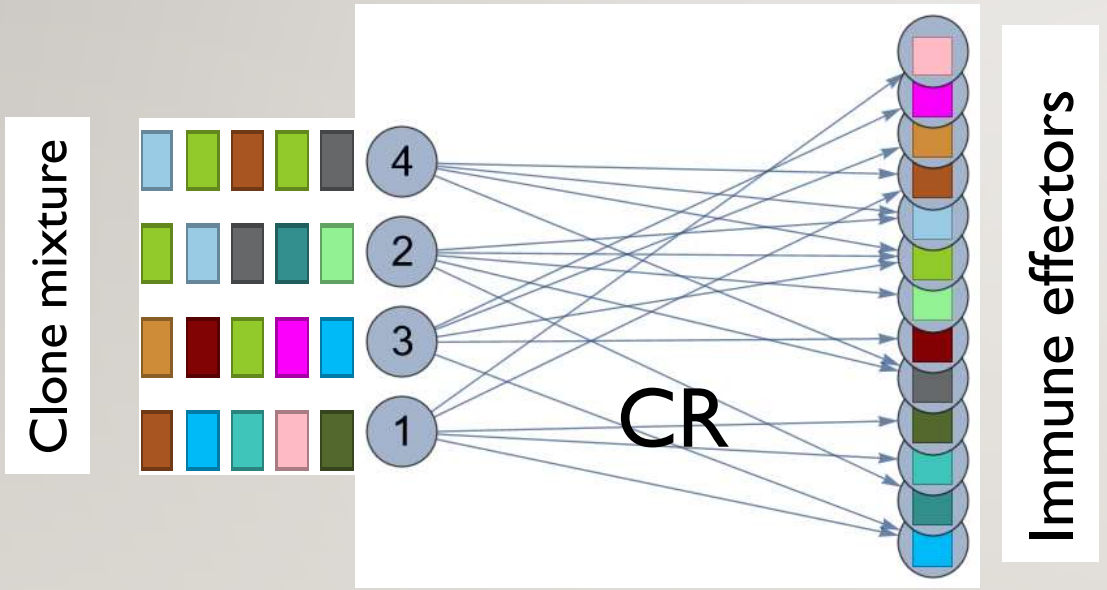


- Crossover (mosquito):

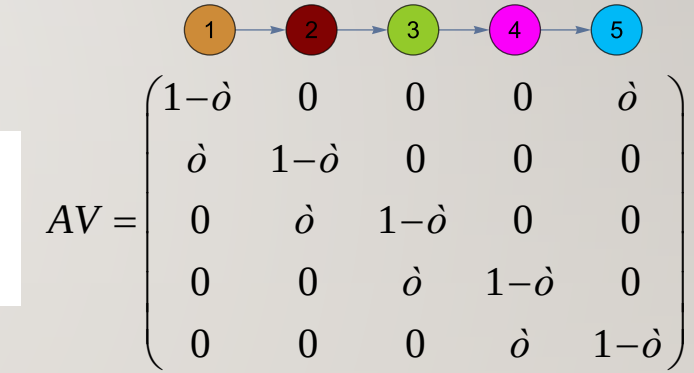
In-host dynamics

In-host state for infecting clones {A,B,…}

Components	RBC	iRBC	Gametocytes	IE (Abs)
Variables	x	$\langle\|A \to \{y_{A1}, y_{A2}, \ldots\}, B \to \{y_{B1}, y_{B2}, \ldots\}, \ldots \|\rangle$	$\{G_A, G_B, \ldots\}$	$\{u_1, u_2, \ldots\}$
input / output	□	inoculae	uptake	□



Sequential AV for 5-Var clone



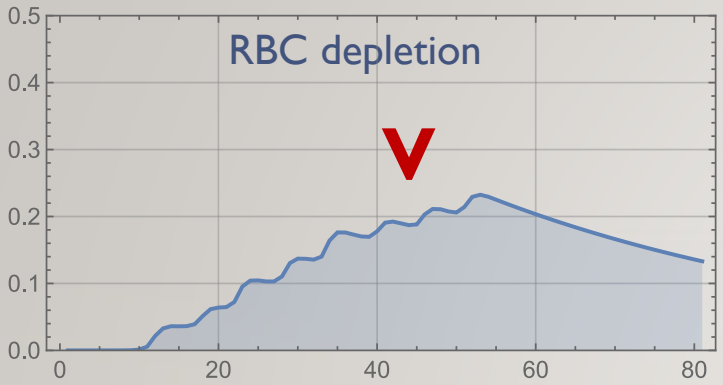
Genotype space ~ m^N
m- samples from (1,2,… N)

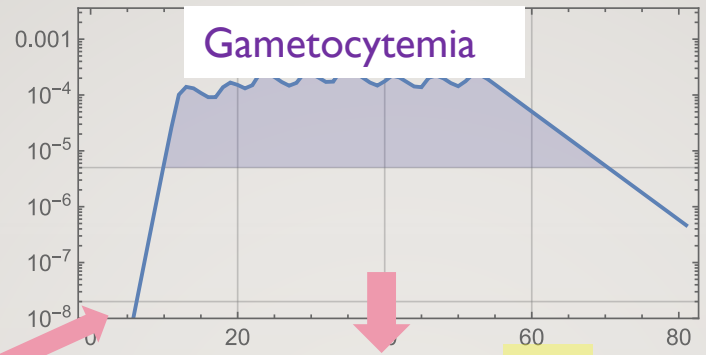
Immune effector space
{1,2,…,N}

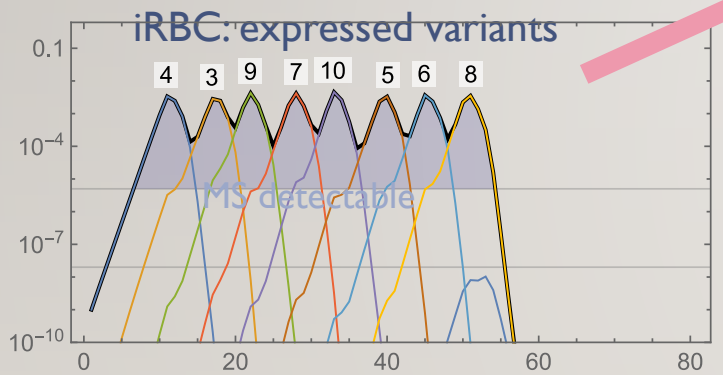
RBC growth/depletion
Immune stimulation, clearing parameters
switching (mutation) rate $\varepsilon = 10^{-5} – 10^{-4} –$

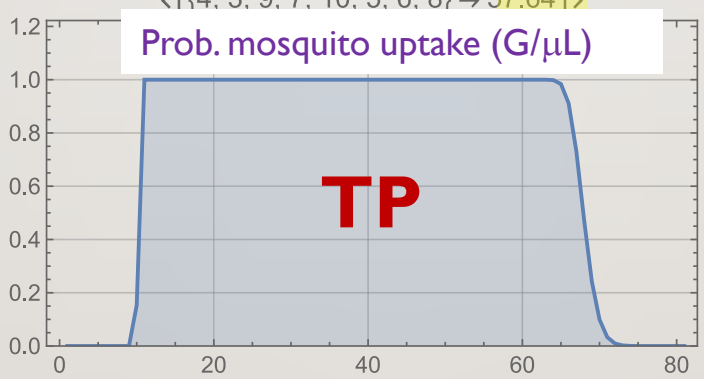
Typical infection history of a single 8-var clone

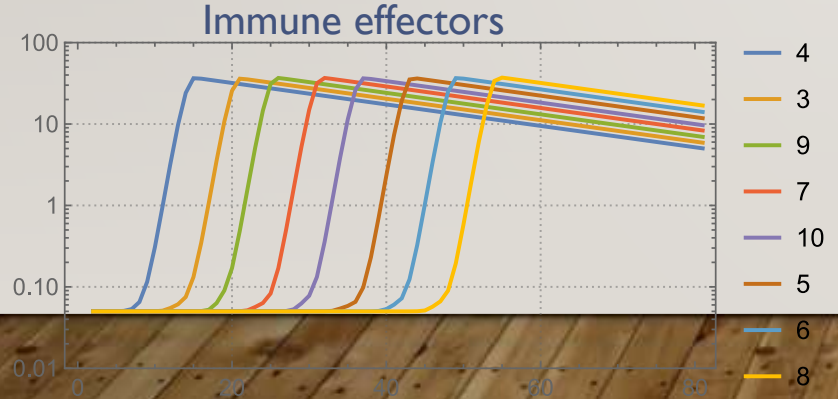
{4,3,9,7,10,5,6,8}



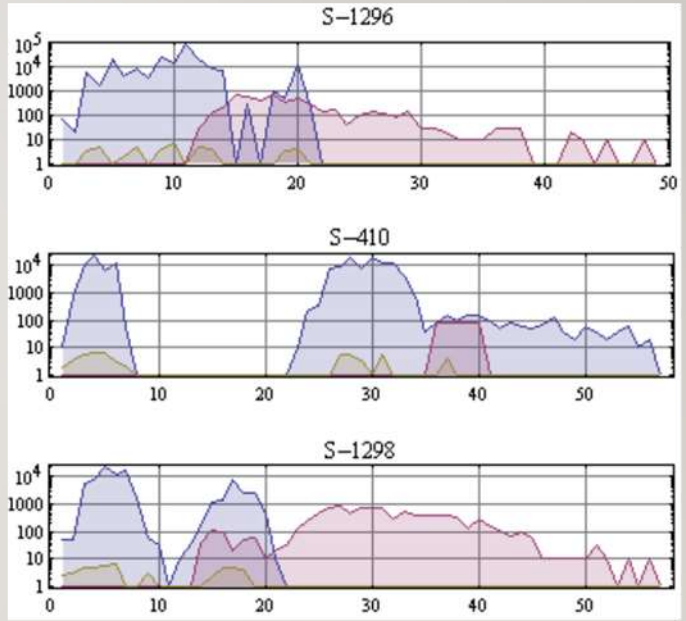








Comparison with Malaria-Therapy data

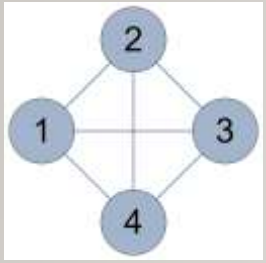


Phenotypes

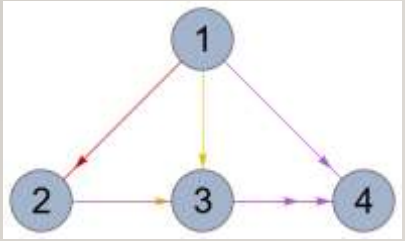
- **V** = RBC depletion
- **TP** = cumulative P-uptake (AUC)

Mixed infections: 4-clone case

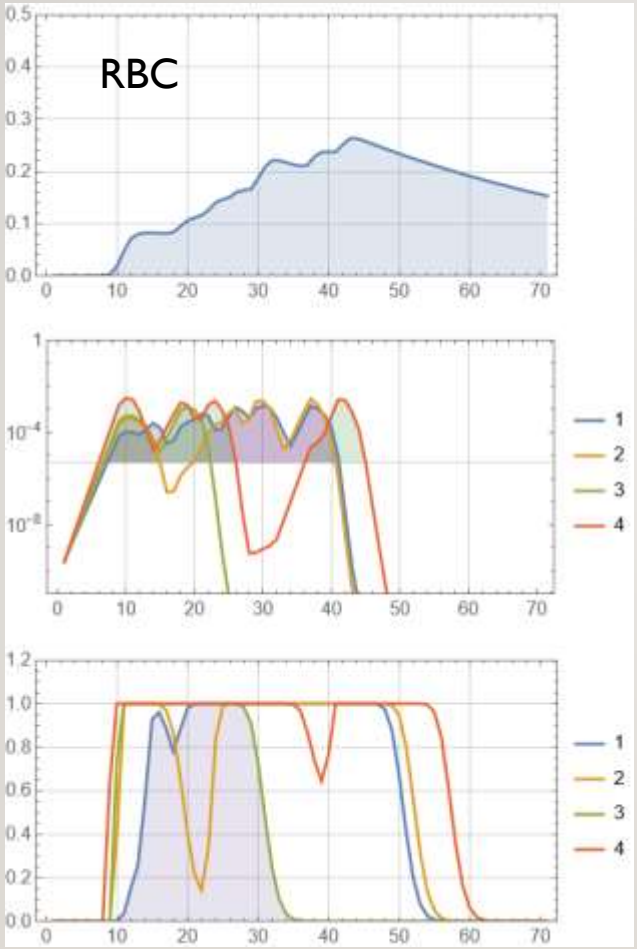
4-mix CR-network



Downstream CR



#1 winner ?
#4 looser ?



TP= AUC - shaded: {1,3}-overlap

Individual-TP outcomes

Equally fit

	"single-clone TP"	"4-mix TP"
1	40.1	36.7
2	41.1	38.6
3	41.5	21.2
4	42.4	47.6

<- Competition ?
<-Cooperation ?

Collective TP outcomes

Single		Pair		Triplet	
		{1, 2}	32.9		
{1}	36.7	{1, 3}	16.9	{1, 2, 3}	13.4
{2}	38.6	{1, 4}	35.6	{1, 2, 4}	31.9
{3}	21.2	{2, 3}	17.2	{1, 3, 4}	16.9
{4}	47.6	{2, 4}	37.6	{2, 3, 4}	17.2
		{3, 4}	21.2		

Cumulative probability of mosquito uptake {i}, {i j}, {i j k)

Complex TP-outcomes of mixed infections not predictable from clonal makeup (CR)

Evolution and selection in host ABM ensembles

Two-level selection: **within-host** (mixed infections) + **community** transmission

I. **Primary selection**: naïve host ensembles

II. **Transmission**:
   - **serial passage** in SP-lines
   - **Host communities** in mosquito environment

Basic questions

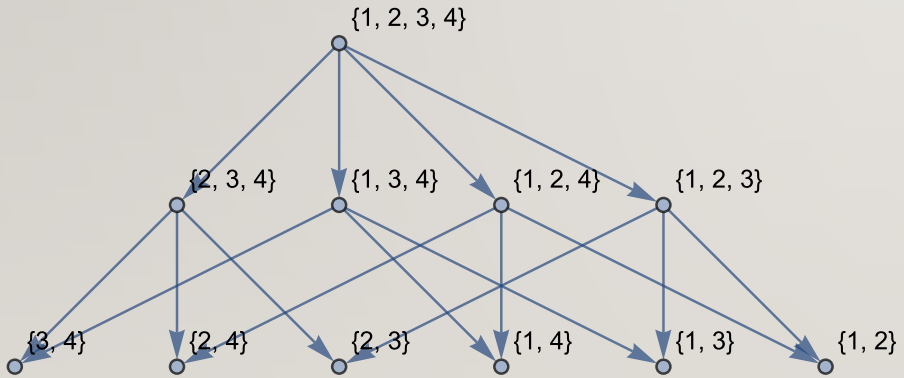
- Which clones or teams (cliques) get selected ?
- Role of **V-TP** phenotypes?
- Role of **transmission environment** (EIR-intensity, mosquito population/ behavior)
- **Control implications**

Primary selection

Identify most fit (TP) individual clones and cooperating teams in mixed contests

Possible setup:

- 5-variant clones 
- **Clone-pool**: 200 random draws {S_i} from gene-space {…  …}
- Nested mixed contests: quintuplets-> quadruples->… -> doublets



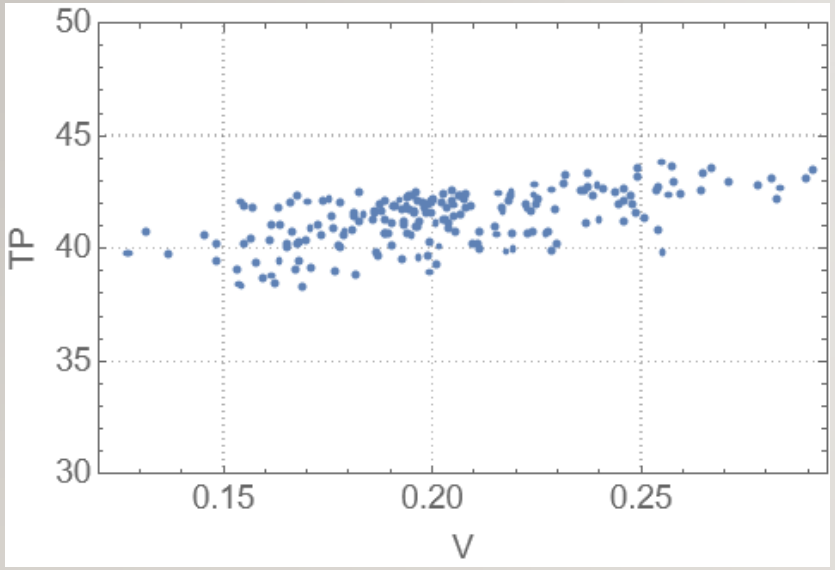
Type	# contests	
double	28490	$SS = \{S_i, S_j\}$
triple	49835	SSS
quadrup	25000	$SSSS$
quintup	5000	$SSSSS$

Contest outputs:

- Individuals- {TP_i}
- couple: {TP_{ij}}
- triplets: {TP_{ijk}}

Statistical analysis : fitness (TP) loss in mixed contests

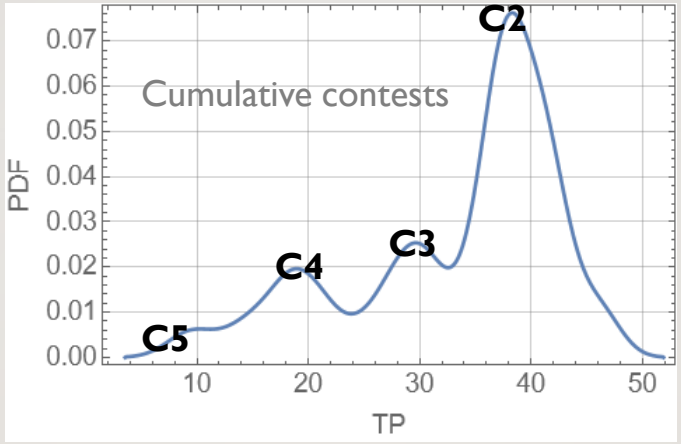
Single-clone infections: V-TP

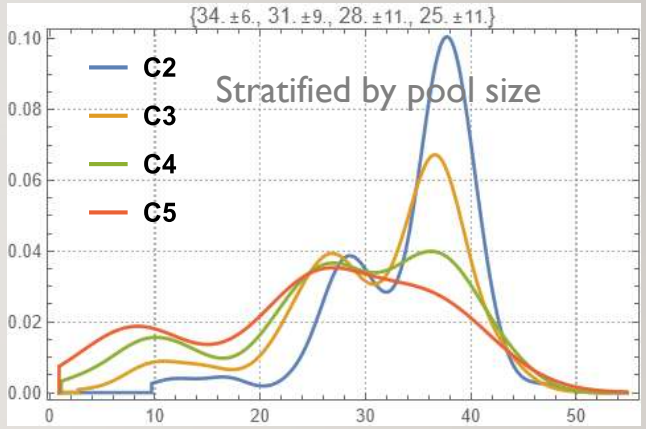


- No **V-TP** correlation
- **V** – minor factor in selection

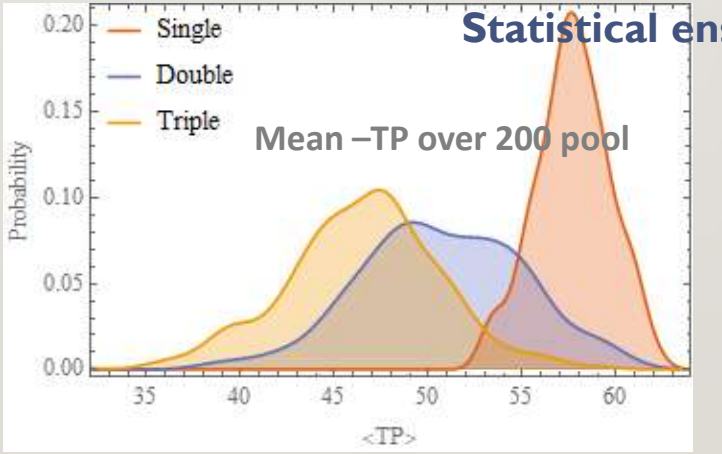
Mixed-infection ensembles

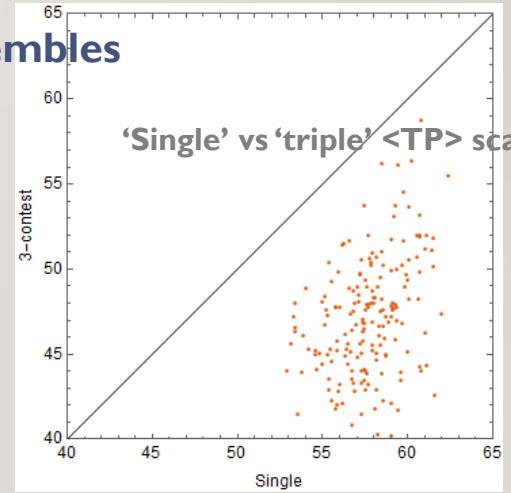
Typical individual TP – distributions



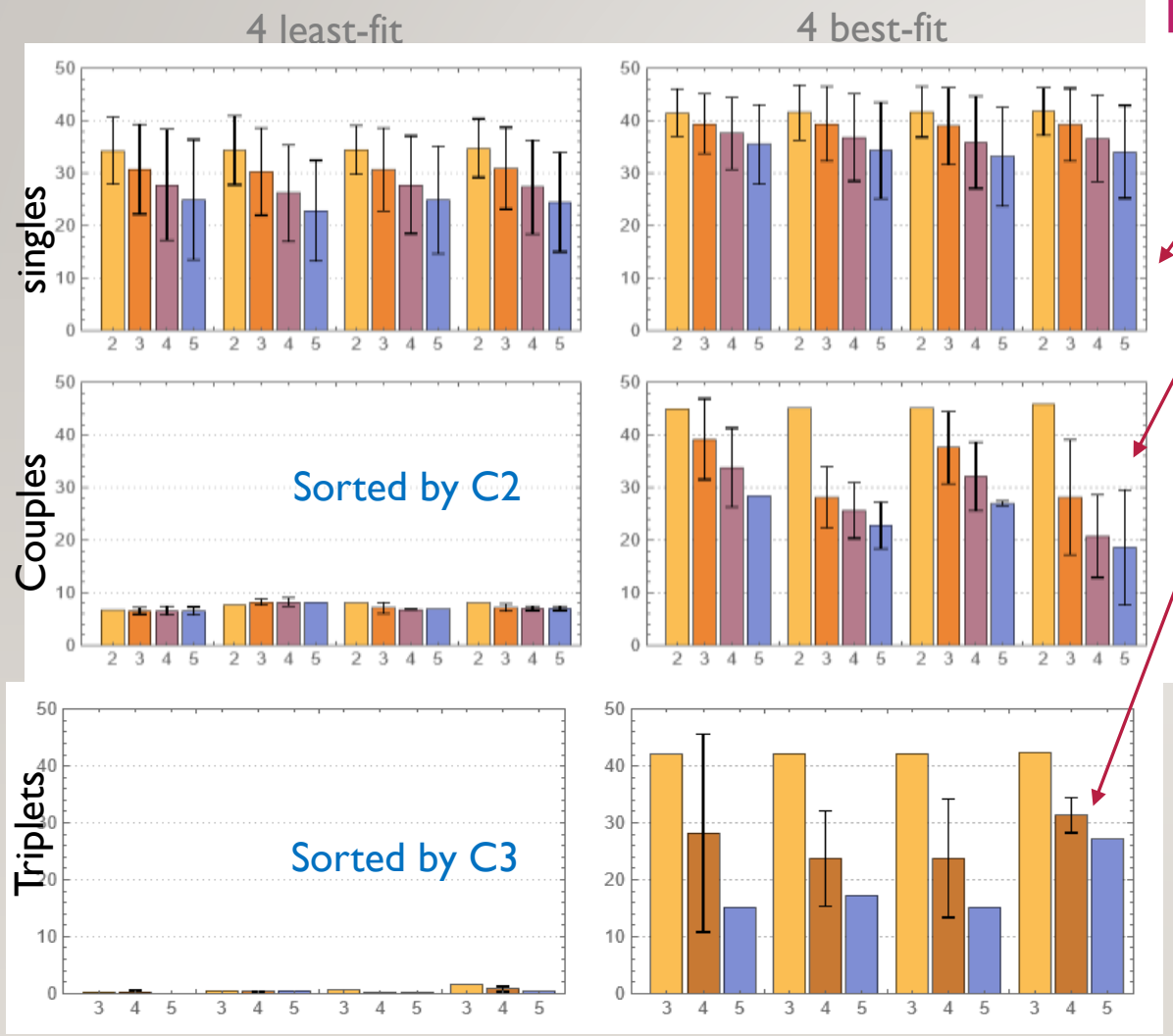


Statistical ensembles



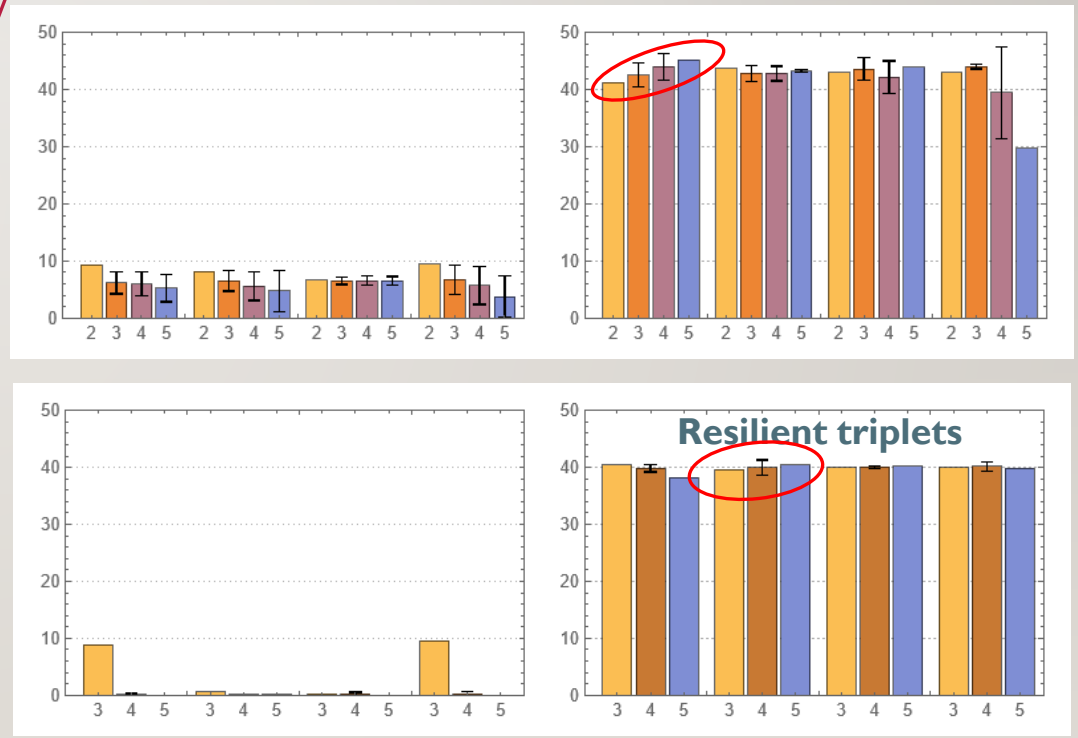


Statistical loss and gain in mixed ensembles



Fitness cost of contest competition

Resilient teams in contest mixtures.



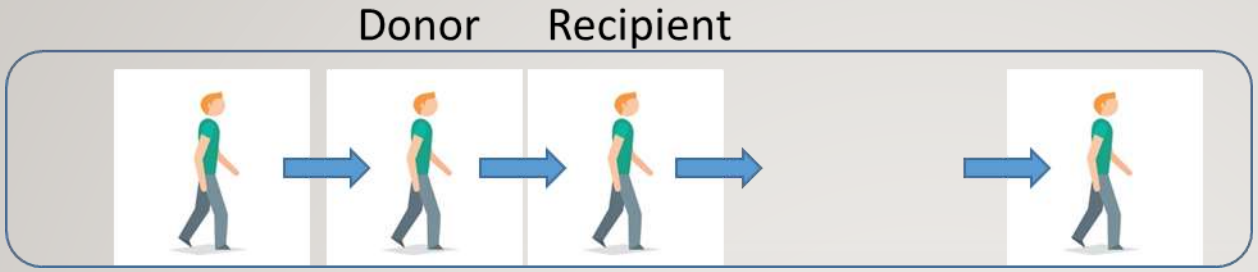
Cooperating cliques ?

More evidence of fitness gain in mixed infections

- Expanded 'couple->triple' $(ij) \rightarrow \{(ijk) : k = 1, 2, ...\}$
- Can triple co-infection improve survival: $\boxed{T_{ij} < T_{ij}}$?
- For most *couples* (of 200 core) 5-30% expanded *triplets* improve TP
- Hence 5-30% chance improve survival via increased mixing

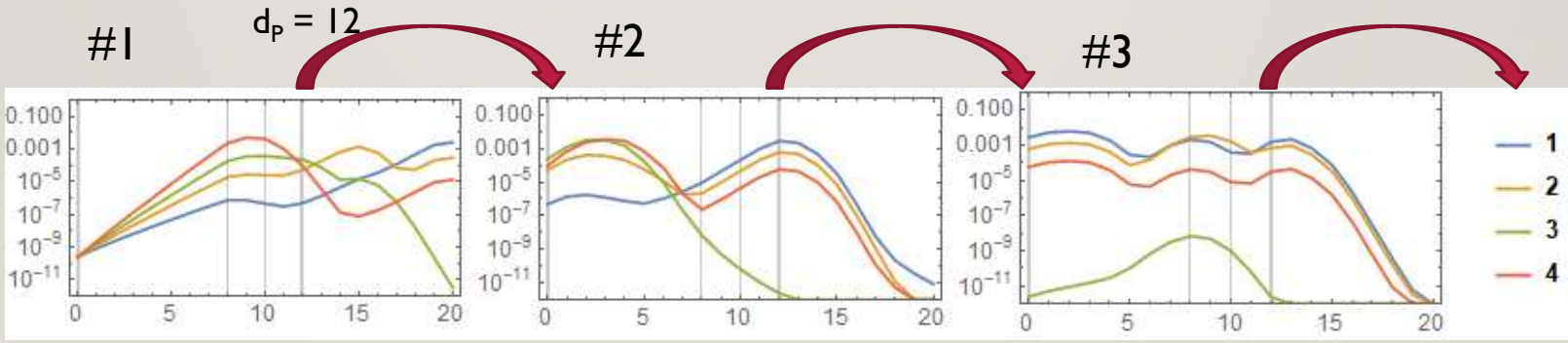
TP	ij	ijk
single	$\{T_i, T_j\}$	$\{T_i, T_j, T_k\}$
mixed	T_{ij}	$\{T_{ij}, T_{ik}, T_{jk}, T_{ijk}\}$

Serial passage (SP) in naïve host lines



- *Strain mixture* $\{S_1, S_2, \ldots\}$ injected in donor #1
- Transmissible strains (gametocytes) collected on *passage day* d_P goes to recipient
- Repeated over multiple cycles, with fixed or random d_P (EIR = transmission intensity)
- Clonal makeup collected over multiple passage cycles

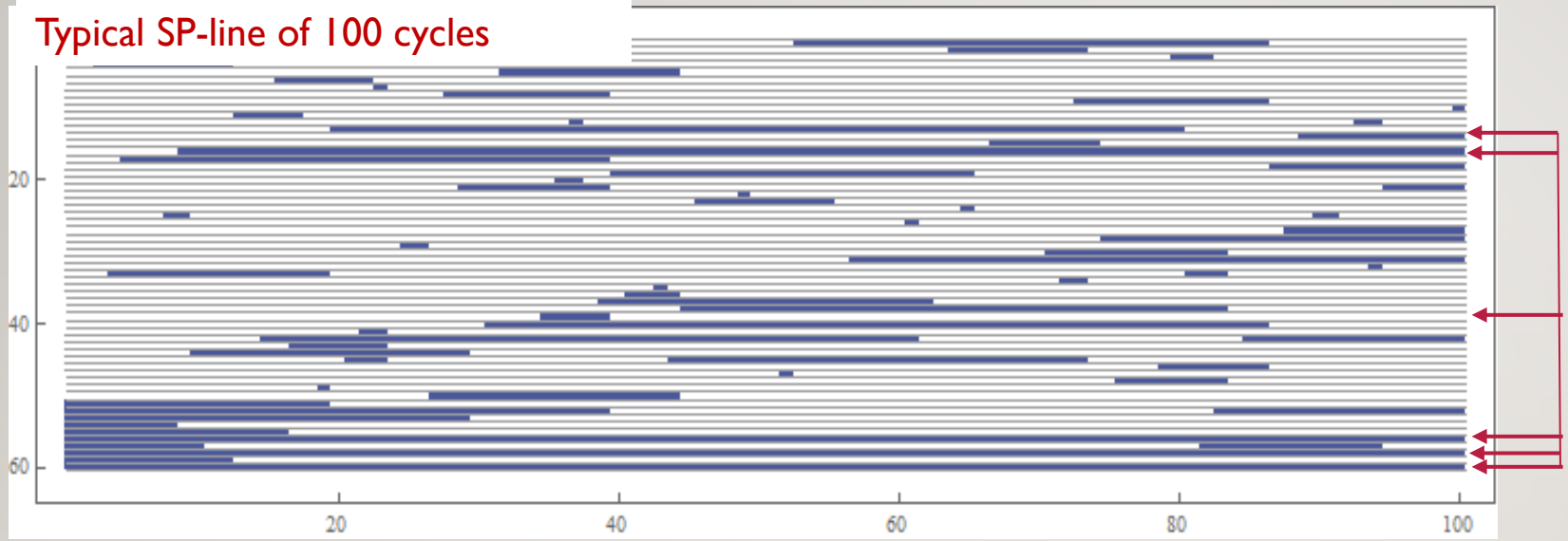
Typical SP line for 4-strain mixture



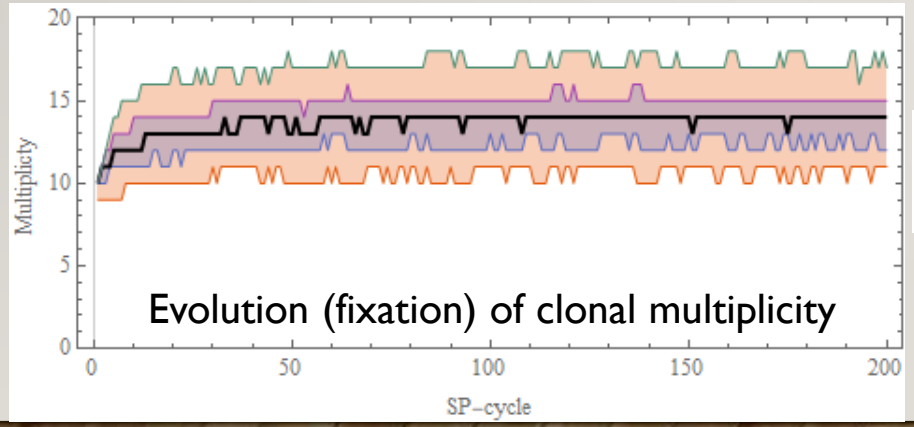
Evolution of SP-ensembles with random infusion

- Ensemble of SP-lines with 100-200 transmission cycles (random dP)
- Initialized with 10 best-quantile of primary selection
- Random infusion of the remaining 190 on each SP-cycle

Typical SP-line of 100 cycles



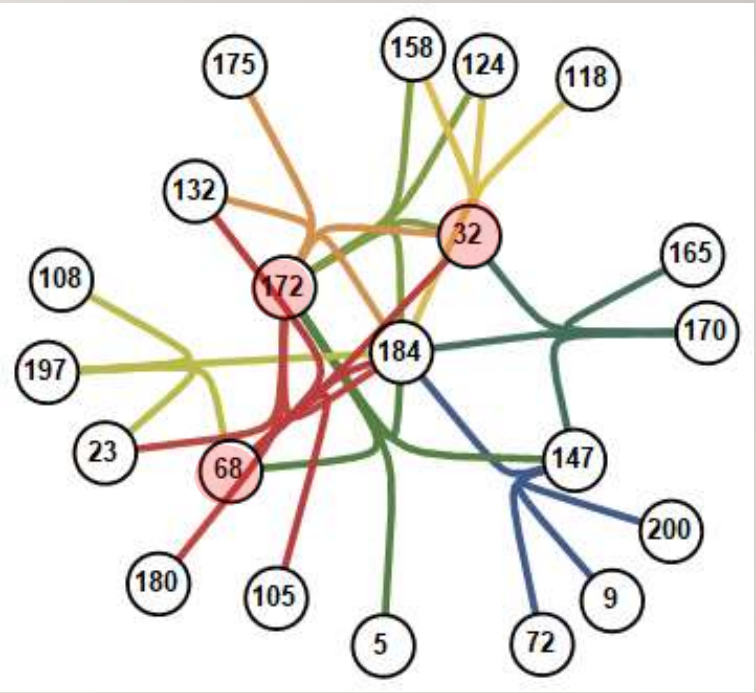
Best survivors



Evolution (fixation) of clonal multiplicity

Multiplicity distribution in multiple SP-lines (SP- ensemble of 200)

Cooperating cliques



Persistent clones engage closely-linked cooperative cliques derived from CR network

Conclusions

- **Methodology**: ABM with genetically structured parasite and detailed in-host biology (target-parasite-immune interactions). Flexible, computationally efficient
- **Intrinsic and derived clonal phenotypes**: virulence (RBC), **TP** (transmission potential
- **Selection** in naïve host ensembles, and SP-transmission lines; fitness (TP) cost of **competition**
- **Key drivers of** selection: clonal cross-reactivity, transmission intensity (EIR, SP frequency
- Evidence of **cooperative behavior** in host ensembles and transmission lines
- **Future work**:
  - Exploration of **cooperating clusters** (cliques) in extended multi-clonal genotype spaces
  - Evolution and selection in **coupled human-mosquito systems with crossover**
  - Implications of **cooperative (persistent) cliques** for monitoring, control, vaccine strategies
- Reference: D. Gurarie, bioRxiv 539676; doi: https://doi.org/10.1101/539676. 2019.