

# Supporting Information

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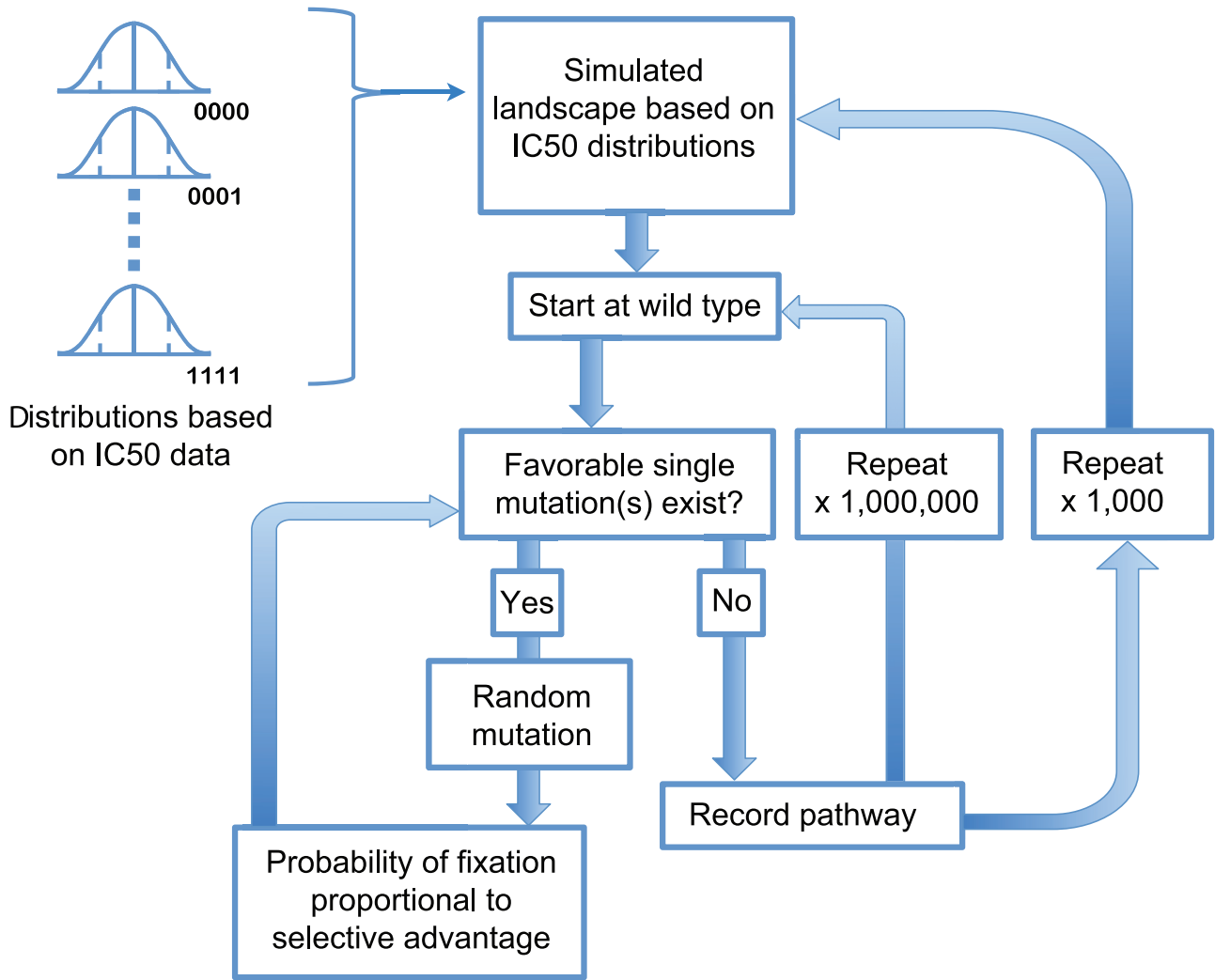


Fig. S1. Flow chart of the algorithm used in the computer simulations.

**Table S1. Comparison of IC<sub>50</sub> and MIC**

Allele	IC <sub>50</sub>	SD	MIC	Rank IC <sub>50</sub>	Rank MIC
0000	0.27	0.04	75	2	4.5
1000	0.36	0.03	25	4	1.5
0100	2.04	0.65	50	5	3
0010	9.56	0.95	150	8	6
0001	0.29	0.05	75	3	4.5
1100	4.89	0.80	425	6	9
1010	37.67	0.58	800	9	11.5
1001	102.67	1.53	800	12	11.5
0110	147.00	3.79	850	13	13.5
0101	7.28	0.37	550	7	10
0011	0.00	0.00	25	1	1.5
1110	242.17	8.47	950	15	15
1101	56.00	1.73	350	10	8
1011	56.83	0.77	325	11	7
0111	195.25	10.05	850	14	13.5
1111	300.12	7.46	975	16	16

IC<sub>50</sub> is the concentration of pyrimethamine that inhibits growth to 50% of its level in the absence of drug; MIC is the minimal concentration of pyrimethamine that completely inhibits growth. Each allele is designated as a vector of 0's and 1's corresponding left-to-right to DHFR amino acid replacements N51I, C59R, S108N, and I164L. At each site, 0 denotes the amino acid at the left (nonmutant), and 1 denotes the amino acid on the right (mutant). Units of IC<sub>50</sub>, the SD of IC<sub>50</sub>, and MIC are all in  $\mu\text{g/mL}$  of pyrimethamine. The IC<sub>50</sub> values in this table are in good agreement with those expected from the  $K_i$  and  $K_m$  values estimated in vitro previously [Sirawaraporn W, Sathitkul T, Sirawaraporn R, Yuthavong Y, Santi DV (1997) Antifolate-resistant mutants of *Plasmodium falciparum* dihydrofolate reductase. *Proc Natl Acad Sci USA* 94:1124—1129].

**Table S2. Growth rates in the absence of pyrimethamine**

Allele	Relative GR	95% CI
0000	1.002	0.117
1000	1.102	0.253
0100	1.193	0.117
0010	1.102	0.171
0001	0.969	0.104
1100	1.822	0.174
1010	0.794	0.071
1001	1.262	0.137
0110	1.149	0.110
0101	1.554	0.265
0011	0.170	0.041
1110	1.429	0.145
1101	1.285	0.226
1011	0.651	0.072
0111	1.274	0.127
1111	0.860	0.082

GR denotes the growth rate of strains carrying each allele, relative to that of a strain carrying the nonmutant allele (0000). Allele designations are as in Table S1. The column headed "95% CI" gives minus/plus deviation from the mean that yields the 95% confidence interval around each estimate.

**Table S3. Normalized mutation matrix for *P. falciparum***

Original nucleotide	Mutant nucleotide			
	A	T	G	C
A	—	0.056	0.049	0.021
T	0.067	—	0.018	0.049
G	0.178	0.109	—	0.050
C	0.090	0.255	0.058	—

Single-nucleotide polymorphisms in isolates of *P. falciparum* were identified using standard neighborhood quality-score criteria relative to the 3D7 reference sequence (base quality score  $\geq$  Q20 for both alleles, no other SNPs within 5 bp), and rooted by assuming that the sequence of *P. reichenowi*, a parasite of chimpanzees, is ancestral. We limited the analysis to intergenic SNPs on the assumption that this sequence class would be least affected by selection and thus would most accurately reflect the profile of new mutations. We identified a total of 1,105 SNPs in intergenic regions of the *P. falciparum* genome for which a high-confidence *P. reichenowi* genotype was available. Observed intergenic SNP counts for each mutational type were scaled according to the overall abundance of the ancestral nucleotide in intergenic regions of the *P. falciparum* genome (A, 43.1%; T, 43.1%; C, 6.9%; G, 7.0%). The scaled mutation rates were then normalized so that the sum of all rates was equal to 1.

**Table S4. Cited sources of polymorphic DHFR alleles in *P. falciparum***

Allele	Location (ref.)
S108N (0010)	Indonesia (1), Philippines (1), Cameroon (2), Burkina Faso (3), Bangladesh (4), Senegal (5), Sudan (6)
N511/S108N (1010)	Kenya (7), Burkina Faso (3), Cameroon (2), Sudan (6), Ethiopia (8), India (9)
C59R/S108N (0110)	Laos (1), Indonesia (1), Thailand (1), Philippines (1), Kenya (7), Cameroon (2), Bangladesh (4), Sudan (6), Iran (10), India (9)
N511/C59R/S108N (1110)	Senegal (5), Sudan (6), Ethiopia (8), Kenya (7), Zambia (11), Benin (12), Cameroon (12), Ivory Coast (12), Gabon (12), Guinea (12), Mali (12), Uganda (12), Thailand (1), Bangladesh (4)
C59R/S108N/I164L (0111)	Bangladesh (4), Iran (10), India (9)
N511/C59R/S108N/I164L (1111)	Bangladesh (4), India (9), Thailand (13)

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