**Fitness model**

Following the fitness model presented in *Krittikorn Kumpornsin et al. MBE. (2014)*, the relative fitness value of *i*th strain parasite (*fi*) is given by [1]

(1.1)

where *a* represents drug activity against drug-sensitive strain (a normalized drug killing rate of drug-sensitive strain). The loss of fitness due to mutations is described by the cost of mutation parameter (*Ci*) and the loss of drug activity due to mutations is described by the efficiency of the resistance mutation (*ei*). All relative fitness values are expressed relative to the fitness of drug-sensitive strain without drug pressure (i.e., *a* = 0, *C*1 = 0, and therefore *f*1 = 1, where *i* = 1 represents the drug-sensitive strain). Our work is to find *Ci* and *ei* using the experimental growth data.

**Parasite growth in the absent of drug**

To find *Ci*, consider the following logistic growth model of parasite in the absent of drug. The number of *i*th strain parasites (*Ni*) is described by

, (1.2)

where is the maximum growth rate of the drug-sensitive strain, *N*max is the carrying capacity, and *N*total is the total population size. If the total population size does not change appreciably, we obtain

, (1.3)

where is an effective growth rate. The solution of equation (1.3) is given by

, (1.4)

where . Using the fact that the cost of mutation of the drug-sensitive strain *C*1 = 0, we obtain

(1.5)

We then performed a curve fit by fitting equation (1.5) to the experimental growth curve of drug-sensitive strain. The fitting result is shown in Figure 1. Note that we use 3D7BC15 as a representative strain of the drug-sensitive strain as its fit has highest R-square.



**Figure 1.** Curve fitting of drug-sensitive strain. Base on the fitting, we found *G* = 0.0523 [95% CI (0.0200 0.0846)] per day with R-square = 0.6613. Error bars show the standard deviation.

Next, we can find *Ci* by fitting equation (1.4) to experimental growth curves of drug-resistant strains (Figure 2 and Table 1).











**Figure 2.** Curve fitting of the other strains.

**Table 1.** Estimated normalized costs of mutation.

|  |  |  |
| --- | --- | --- |
| **Strain** | **Normalized cost of mutation** | **R-square** |
| 3D7BC24 | 0.0327 [95% CI (-0.0462 0.1116)] | 0.3876 |
| 3D7BC26 | 0.0558 [95% CI (-0.8884 1.0000)] | 0.0092 |
| Kh2BC7 | 0.0807 [95% CI (-0.2452 0.4067)] | 0.1663 |
| V1SBC1 | 0.2619 [95% CI (-0.1799 0.7036)] | 0.7699 |
| V1SBC23 | 0.1822 [95% CI (-0.1406 0.5051)] | 0.7489 |

**Parasite growth under drug pressure**

To find the efficiency of the resistance mutation (*ei*) and the drug activity (*a*), consider the following model of parasite growth under drug pressure [1-3]

, (1.6)

, (1.7)

where *ki* is a rate at which *i*th strain parasites are kill by a drug dose *D*. *K* is the maximal killing rate, *A* is drug affinity, and *H* is the Hill coefficient, which determines the steepness of the killing rate as a function of effective drug dose. We may choose *H* = 1, which is representative of many drugs.

We can find *K* by fitting equation (1.6) to the experimental growth data of the drug-sensitive strain at a very high drug concentration (500 nM), in which *k*1 ≈ *K*. The fitting results are shown in Figures 3 - 5.



**Figure 3.** Growth curve of 3D7BC15 at 500 nM of CQ. Based on the curve fitting, the maximum killing rate for CQ is 0.7870 [95% CI (0.5989 0.9751)] per day with R-square = 1.0000.



**Figure 4.** Growth curve of 3D7BC15 at 500 nM of PPQ. Based on the curve fitting, the maximum killing rate for PPQ is 0.1514 [95% CI (0.0247 0.2780)] per day with R-square = 0.9982.



**Figure 5.** Growth curve of 3D7BC15 at 500 nM of MQ. Based on the curve fitting, the maximum killing rate for MQ is 0.1288 [95% CI (-0.1718 0.4295)] per day with R-square = 0.9776.

Next, by using experimental growth data of the drug-sensitive strain under drug pressure and the computed value of *K*, we can find *A* using equation (1.7) as follows:

(1.8)

Note that we have used the fact that *e*1 = 0. The results are shown in Figures 6 – 8.



**Figure 6.** Growth curve of 3D7BC15 at 50 nM of CQ. Based on the curve fitting, the drug affinity for CQ is 32.0175 nM [95% CI (16.0641 47.9708)] with R-square = 0.9980.



**Figure 7.** Growth curve of 3D7BC15 at 50 nM of PPQ. Based on the curve fitting, the drug affinity for PPQ is 214.0637 nM [95% CI (-305.8037 733.9312)] with R-square = 0.9954.



**Figure 8.** Growth curve of 3D7BC15 at 10 nM of MQ. Based on the curve fitting, the drug affinity for MQ is 38.5977 [95% CI (-171.2363 248.4316)] nM with R-square = 0.6806.

Finally, by using experimental growth data of drug-resistant strains, *ei* can be calculated using equations (1.7) as follows

(1.9)

where *ki* is calculated by fitting equation (1.6) to the experimental growth data of the drug-resistant strain. The results are shown in Tables 2 - 4.

**Table 2.** Estimated normalized efficiency of the resistance mutation for CQ.

|  |  |  |
| --- | --- | --- |
| **Strain** | **Normalized *ei*** | **R-square** |
| 3D7BC24 | 0.0364 [95% CI (-2.2904 2.3632)] | 0.9979 |
| 3D7BC26 | 0.1193 [95% CI (-7.6412 7.8798)] | 0.9795 |
| Kh2BC7 | 0.5000 [95% CI (-2.4568 3.4568)] | 0.9495 |
| V1SBC1 | 0.3851 [95% CI (-4.0419 4.8121)] | 0.8952 |
| V1SBC23 | 0.4564 [95% CI (-2.8251 3.7378)] | 0.1202 |

**Table 3.** Estimated normalized efficiency of the resistance mutation for PPQ.

|  |  |  |
| --- | --- | --- |
| **Strain** | **Normalized *ei*** | **R-square** |
| 3D7BC24 | 0.0975 [95% CI (-0.1945 0.3895)] | 0.2108 |
| 3D7BC26 | 0.1756 [95% CI (-1.3886 1.7397)] | 0.0079 |
| Kh2BC7 | 0.5000 [95% CI (-0.4369 1.4369)] | 0.6688 |
| V1SBC1 | 0.0000 [95% CI (-1.0175 1.0175)] | 0.9882 |
| V1SBC23 | 0.3444 [95% CI (-0.4106 1.0993)] | 0.3369 |

**Table 4.** Estimated normalized efficiency of the resistance mutation for MQ.

|  |  |  |
| --- | --- | --- |
| **Strain** | **Normalized *ei*** | **R-square** |
| 3D7BC24 | 0.4559 [95% CI (0.3897 0.5221)] | 0.3990 |
| 3D7BC26 | 0.5000 [95% CI (-0.0254 1.0254)] | 0.8185 |
| Kh2BC7 | 0.4337 [95% CI (0.2309 0.6366)] | 0.8075 |
| V1SBC1 | 0.0466 [95% CI (-0.9000 0.9932)] | 1.0000 |
| V1SBC23 | 0.0000 [95% CI (-1.0204 1.0204)] | 0.9999 |

The fitness of each strain relative to the fitness of 3D7BC15 in the condition without drug pressure is obtained using equation (1.1.) where the drug activity against drug-sensitive strain or a normalized drug killing rate of drug-sensitive strain (*a*) is given by

. (1.10)

Figures 9 - 11 show graphs of the fitness as a function of drug concentration.



**Figure 9.** Relative fitness under CQ. The y-axis represents the fitness (relative to the fitness of 3D7BC15 in the condition without drug pressure). The x-axis represents the CQ concentration in unit of nM. Notice that the fitness of both drug-sensitive and drug-resistant strains decreases as the concentration increases. However, the fitness of the drug sensitive strains is more sensitive to the increase in drug concentration.



**Figure 10.** Relative fitness under PPQ. The y-axis represents the fitness (relative to the fitness of 3D7BC15 in the condition without drug pressure). The x-axis represents the PPQ concentration in unit of nM.



**Figure 11.** Relative fitness under MQ. The y-axis represents the fitness (relative to the fitness of 3D7BC15 in the condition without drug pressure). The x-axis represents the MQ concentration in unit of nM.

**Selection coefficient**

In population genetics, a selection coefficient is a measure of differences in relative fitness [4]. The selection coefficient of the *i*th strain parasite under the drug concentration *c* can be defined as

The selection coefficient is a measure of the fitness of the *i*th strain parasite, when they are exposed to the drug concentration *c*, relative to that of the wild-type parasite. If is positive, this implies that the *i*th strain parasite is less fit than the wild-type parasite. In contrast, if is negative, the *i*th strain parasite is more fit than the wild-type parasite. The selection coefficients of parasites under the CQ, PPQ, and MQ are shown in Figures 12-14, respectively.



**Figure 12.** The selection coefficients under CQ.



**Figure 13.** The selection coefficients under PPQ.



**Figure 14.** The selection coefficients under MQ.

**References**

[1] Kümpornsin, K. et al. Origin of robustness in generating drug-resistant malaria parasites. Molecular Biology and Evolution. Volume 31, Issue 7, July 2014, Pages 1649-1660.

[2] Torella, J.P., Chait, R., Kishony, R. Optimal drug synergy in Antimicrobial Treatments. PLoS Computational Biology. Volume 6, Issue 6, June 2010, Pages 1-9.

[3] Pimenta, F. et al. What should be considered in the treatment of bacterial infections by multi-drug therapies: A mathematical perspective? Drug Resistance Updates. Volume 17, Issue 3, July 2014, Pages 51-63.

[4] Gillespie, John H. (2004). Population genetics: a concise guide (2nd ed.). Baltimore, Md.: Johns Hopkins University Press. ISBN 0801880092.