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The dynamics of mutations associated with anti-malarial drug resistance in *Plasmodium falciparum*

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Abstract

The evolution of resistance in *Plasmodium falciparum* against safe and affordable drugs such as chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) is a major global health threat. Investigating the dynamics of resistance against these antimalarial drugs will lead to approaches for addressing the problem of resistance in malarial parasites that are solidly based in evolutionary genetics and population biology. Here we discuss current developments in population biology modeling and evolutionary genetics. Despite great advancements achieved in the past decade, understanding the complex dynamics of mutations conferring drug resistance in *P. falciparum* requires approaches that consider the parasite population structure among other demographic processes.

Malaria control strategies

Malaria is endemic in most of the tropical and subtropical ecosystems, worldwide, and exhibits great ecological and epidemiological diversity [1]. Current malaria control strategies are based on: (i) case management (diagnosis and treatment); (ii) infection prevention (vector control with insecticide-treated bed nets, indoor residual spraying of insecticides); and (iii) disease prevention (e.g. intermittent preventive treatment of pregnant women). These methods are, and will continue to be, the foundations of malaria control and elimination programs.

Unfortunately, resistance to the most commonly used antimalarial drugs emerged, worldwide, in *Plasmodium falciparum*, and render these drugs useless in many endemic areas [2].

Currently, most countries are using artemisinin-based combination therapies (ACTs); however, there are concerns that resistance against these drugs is already beginning to evolve [3].

Although epidemiological surveillance of drug resistance is an important element in the 'war on malaria' [4], the real challenge is to extend the useful therapeutic life of antimalarial drugs by reducing the probability of resistance emergence and spread. We know that the problem is not 'whether' resistance will emerge, but rather 'when' and 'how' such an event will take place. Even though our ability to predict the emergence and spread of resistance prospectively is quite limited, we can make policy decisions that might delay the emergence of ACT resistance in a generic parasite population. The scientific framework supporting such policies comes from mathematical models and empirical data on the emergence of resistance to chloroquine (CQ), sulfadoxine-pyrimethamine (SP), and other antimalarial drugs. Unfortunately, two major

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problems are: (i) some of the frameworks used to generate empirical data cannot be easily incorporated into available population dynamic models; and (ii) the complex geographic structure and evolutionary history of *P. falciparum* have not been properly considered.

In this review, we will outline the need to develop more complex models that consider the spread of drug resistance in a geographically structured population such as *P. falciparum*. Such models should also consider the wide spectrum of clinical disease that determines drug use against *falciparum* malaria. As a first step in developing such models, we need to characterize the parasite populations by incorporating population genetic concepts.

Parasites populations: act locally but think globally

Within a classical ecological and epidemiological framework, the parasite population dynamics are described in terms of the number of infections that co-occur, in space and time, in a well-mixed population of susceptible hosts. Under such an approach, drug selection is represented in terms of differential transmissibility (different values of the reproductive rate, R_0 [5] or different numbers of secondary infections) of resistant and sensitive genotypes. The response variables of interest are the incidence, prevalence, and frequency of infections with drug resistant parasites (see, for example, Ref. [6]).

Under this ecological and epidemiological paradigm, the outcomes of models assessing the success of a given malaria treatment policy depend, along with other epidemiological factors, on the local transmission, case management, and drug use. This approach is useful if we assume that: (i) resistance is already present in this single well-mixed population; (ii) the advantage of resistant genotypes can be assumed constant; (iii) back mutations turning resistant into sensitive parasites are unlikely; (iv) migration does not affect the frequency of drug-resistant mutations; and (v) the population is large enough to ignore random extinction or fixation of mutations. In spite of this large set of simplifying assumptions, these models can provide data that are useful for the planning of local malaria control programs. This approach, however, provides limited information if we try to predict whether drug resistance will originate and how it will disperse at its early stages when resistant mutations are in low frequency. Such assessments are required in order to build up a model from a local to a regional or global level where comparisons are needed across different populations, probably with distinct ecologies and evolutionary histories [7,8]. These aspects are addressed by population genetic models. However, while the available models have allowed, for example, changes in the strength of selection [8], they are still limited in terms of explicitly considering the geographic structure or any other form of population structure (departure from random mating). This geographic structure, together with epidemiological factors will shape how drug selection operates on these newly emerging or recently introduced (but still rare) mutations associated with drug resistance; precisely at the moment when we need to contain them. The geographic structure is the result of the evolutionary history of the parasite populations in terms of migration patterns, temporal and spatial differentiation, and differences in their effective population sizes. Although the importance of migration and geographic differentiation (structure) is relatively easy to grasp, it is far less intuitive that there is 'an effective population' size affecting how natural selection acts on a mutation in a given population [9].

The effective population size (N_e) represents the number of random mating parasites in an ideal population that would maintain the same amount of genetic variation observed in an actual population with a given census size [9], N (direct count of parasites in all infected hosts). The effective population size thus measures how random genetic drift will affect the allele frequencies in a given population. At small N_e , stochastic fluctuation of genotype frequencies (i.e. genetic drift) can lead to the loss or fixation of genotypes, regardless of their fitness [8, 9]. Therefore, genetic drift may greatly reduce the efficacy of selection (measured by the

selection coefficient [s] on mutations that affect the fitness, when such mutations are in low frequency. In large populations, however, selection is more important than genetic drift to explain the dynamic of mutations that affect the fitness [9]. Since the effectiveness of selection versus genetic drift is measured by $N_e s$ [9], what constitutes a ‘large’ or a ‘small’ effective population size depends on the strength of selection, s .

The unique mating system of the malaria parasite contributes to reducing drastically the effective population size. Indeed, the number of blood-stage parasites that generate gametocytes, conform diploid oocysts, and then are transmitted over different hosts is much smaller than the total direct count of haploid parasites among all infected hosts, causing $N_e \ll N$ [8]. Among other factors, the failure to recognize this difference led to the expectation that drug resistance would successfully disperse from several independent origins on numerous occasions, but a surprisingly relatively small number of genetic backgrounds have been observed globally in association with resistance mutations (see Box 1, Figure 1a). This pattern offered a first glimpse into the importance of geographic structure in the dispersion of drug resistance.

While addressing this global geographic level is important, it does not account for local differences in effective population size, migration patterns, selection, and transmission that are useful whenever we want to contain the dispersion of drug-resistant mutations at a local-regional level. Unfortunately, there are only a few studies that consider the parasite population structure at a local-regional scale along with the strength of selection due to drug pressure in each population [27]. Most researchers have focused on single populations, arbitrarily defined, and sometimes on single genes [16,20,28-30]. There is extensive epidemiological data examining the frequency of drug-resistant mutations in areas with different levels of malaria transmission and drug use [15,18,19,21]. However, these studies usually do not consider the underlying evolutionary and ecological processes that contribute to the observed distribution and frequency of resistant mutants.

Overall, selection operates on mutations associated with drug resistance at temporal and spatial scales that are usually poorly defined and seldom measured. Understanding such scales, or at least representing them with some reproducible population genetic metric, will allow us to incorporate our knowledge about the dynamics of mutations associated with drug resistance into malaria control and eradication programs. Building bridges between population dynamic models and population genetic models that consider such geographic complexity remains as a challenge for those modeling malaria drug resistance.

Inferring the strength of drug selection

Regardless of the biological differences in how resistance against antimalarial drugs has built up [4], several alleles conferring some degree of drug resistance segregated simultaneously, worldwide [22]. Yet, different outcomes are possible when we consider any particular subpopulation at the local level: (i) one allele might go to fixation; (ii) several adaptive alleles might co-exist if they are favored under different conditions; or (iii) several alleles could increase in frequency and might perhaps be adaptively equivalent in a given ecological setting allowing their relative frequencies to be determined by genetic drift. Discriminating scenarios in populations that are spatially structured might be relatively easy if drug selection is somehow constant and we can wait until all populations reach genetic equilibrium. Equilibrium, in a population genetic sense, is the state at which the allele distribution and the frequencies of mutations are constant over time. Such equilibrium is unlikely to be achieved in reality, e.g. it is not a viable policy to keep using ineffective drugs until resistance is totally fixed in the parasite population or to control human movements so the effect of migration on parasite populations remains constant.

The situation is more complex if we consider that changes in drug use also affect the relative fitness of these mutations, as research on SP and CQ resistance has shown. These mutations evolved multiple times under strong drug pressure (see Box 1, Figure I); however, our current knowledge also indicates that there is a fitness cost associated with these mutations in the absence of drug pressure [8,22]. Malawi prohibited the use of CQ back in 1993, but CQ-sensitivity has been recovered after several years [31] as result of the expansion of wild-type strains rather than back mutations from CQ resistant haplotypes [32]. In the case of SP, there is evidence that sensitivity to the drug is increasing in Peru [33].

In order to support drug-use policies, we want to predict outcomes when drug-resistant mutations appear and are still in low frequency, those are precisely non-equilibrium conditions. Yet, such non-equilibrium conditions have not been properly addressed by the population genetic models available [8]. This non-equilibrium dynamics determines the establishment of advantageous mutations such as those associated with drug resistance, so it cannot be ignored [34-36]. In addition, environmental heterogeneity can affect the emergence of advantageous alleles. For example, Hadany [37] showed that evolution due to jointly advantageous mutations is faster in subdivided populations with heterogeneous selective pressures among demes. Such heterogeneous selective pressures could, in the case of malaria drug resistance, be the result of differences in treatment guidelines between countries where there is gene flow among parasite populations, decoupled transmission seasons at a given spatial scale [38], seasonality in transmission [38,39], and the presence of individuals with acquired natural immunity that are asymptomatic and do not require treatment [40]. All these levels of heterogeneity will affect the fitness of mutations associated with drug resistance, although quantifying such fitness then is a laborious endeavor.

Traditional approaches used experimental systems to explore the potential differences among alleles in the presence and absence of the drug in order to make inferences about their fitness [41,42]. Although experimental systems are valid first approximations, they are not the measurements of the fitness of resistant parasites in natural populations because they ignore the environment where mutants are expressed. A second approach requires longitudinal epidemiological and genotyping data derived from an arbitrarily defined population [43]; however, such estimates inevitably sum the effects of several local processes, including migrations. Unfortunately, longitudinal epidemiologic and genotyping data are not widely available and we cannot simply extrapolate from inferences made on a single population to a global level assuming a universal relative fitness. A promising approach uses the size of the genome regions affected by genetic hitchhiking in a sample of alleles when advantageous mutations are sweeping through the population [27,44].

Resistant mutations selected by antimalarial drugs remove linked neutral variation as they sweep (increase in frequency) through a parasite population; a process called 'genetic hitchhiking' [45]. The genomic region affected by such processes is characterized by (i) a reduction of genetic variation in the regions surrounding the selected locus [45,48], (ii) an increment in linkage disequilibrium (LD) between the selected mutation and flanking sites [49,50], and (iii) a skewed distribution of allele frequencies at loci nearby on the chromosome [51,52]. Such a genetic footprint can be used to assess the strength of selection on a given set of mutations [44] under a variety of epidemiological and evolutionary contexts in the absence of longitudinal epidemiologic and genotyping data, thus, inferences about the unknown evolutionary history of drug-resistant mutations can be made. However, there are still challenges to using such approaches.

The patterns generated by selective sweeps are better understood in the case of a single mutation that increases in frequency in a closed population with random mating [47]. However, it has been shown with SP and CQ that several resistant alleles segregate in a population

simultaneously [22], and we know that *P. falciparum* populations are poorly described by the single random mating population model [7,23-25]. Thus, more empirical and theoretical research is needed. Such research could focus on resistance to SP and CQ as case studies to better understand and detect patterns of genetic diversity associated with drug resistance. Relatively few studies, however, have shed light on how local demographic processes and geographic structure might affect the extent of the genome region subject to genetic hitchhiking in malaria parasite populations [17,20,25,30]. Even more important, little effort has been made in relating such genetic footprints with existing population dynamics and population genetic models.

Drug selection and transmission intensity

Population structures defined as the patterns emerging from non-random mating are more complicated than simply considering populations differentiating, because they are genetically isolated by distance (geographic structure). An aspect that deserves special consideration is the observed differences in transmission intensity. In areas of high transmission intensity, the host will have more polyclonal infections and, consequently, will have a higher probability of transmitting gametocytes of multiple genetic backgrounds that will recombine in the mosquito. Overall, these populations with high transmission exhibit less linkage disequilibrium [7,25]. This is particularly important when resistance is still rare and encoded by more than one locus. In low transmission settings, the scenario is exactly the opposite: there is less recombination and a more clonal population structure due to inbreeding. As a result, the populations exhibit more LD [7,25]. Such high inbreeding will also directly reduce the number of independent gametes participating in reproduction and, consequently, the effective population size [53-55]. Reduced effective population sizes due to low recombination rates will affect how selection acts on mutations associated with antimalarial drug resistance. Drug-resistant mutations, for example, could go to fixation by chance alone, even after drug pressure is eliminated, if they are already in high frequency. Indeed, drug-resistant genotypes can become fixed and persist in isolated populations, even after the termination of drug pressure as has been observed in some areas in Venezuela for SP-resistant mutations [17].

The intensity of transmission also determines heterogeneous drug selective pressure in *P. falciparum* populations [38,40]. Individuals in areas of low transmission usually have low or no acquired immunity and those in areas of high transmission have and maintain acquired immunity due to continuous re-infections. This acquired immunity determines whether an individual shows signs of clinical disease and needs drug treatment (i.e. the selection pressure), a key factor in the development of resistance [56]. Areas of low transmission, the argument follows, will have a greater proportion of parasites encountering a drug and are under greater selection pressure than areas of high transmission (see Box 1, Figure 1b). By contrast, areas of high transmission will have a high proportion of asymptomatic cases, increasing the environmental heterogeneity where drug selection operates. Despite significant reductions in clinical symptoms and infectiousness, immune individuals still become infected and remain infectious to mosquitoes, even in holoendemic areas, and thus contribute to the parasite population [57,58]. Thus, immune asymptomatic individuals will affect the fitness landscape of mutations associated with drug resistance, allowing the persistence of sensitive alleles [40]. Overall, the dynamic of drug-resistant mutations might be poorly represented by models that ignore the proportion of immune individuals and the seasonality of malaria transmission [40,59].

Modeling drug resistance: lighting a candle in a dark room

Nowadays, mathematical models of antimalarial drug resistance have been based on either an epidemiological (i.e. population dynamic) or a population genetic approach, although complex

simulation models are also being used [60]. The population dynamic approach builds on malaria models that were first introduced by Ross [61] and MacDonald [62]. The first epidemiological model for the evolution of antimalarial resistance was developed by Koella and Antia [6]; the assumptions about resistance were based on a model for the evolution of drug resistance in bacteria [63]. Koella and Antia concluded that immunity was not relevant for the evolution of resistance, in part, because they assumed immunity was complete. When there is partial immunity and semi-immune individuals participate in transmission, albeit at a reduced rate, immunity does affect the fitness of resistant parasites and the evolution of resistance [40]. Koella and Antia also considered superinfection by allowing for a host to carry both drug-resistant and drug-sensitive phenotypes. However, it was later demonstrated that this implementation of superinfection was unrealistic because it allowed co-existence of phenotypes under mathematical conditions that were not biologically realistic [64]. Because superinfection plays an important role in both competition and parasite out-crossing, this remains an important area for future research.

On the side of population genetic models, there have been two major applications. First, the basic population genetic theory of mutation, selection, and recombination was used to understand the general forward-in-time dynamics of drug resistance. Assuming demographic equilibrium, population genetic modeling has been incorporated in an ecological and mathematical epidemiology framework [8,56,64] to elucidate the fitness consequences of resistance by considering transmission intensity, MOI, and human immunity [40,56,59,64]. These models, however, do not consider how selection by antimalarial drugs and the emergence of resistance are affected by the parasite population structure. Regardless of these limitations, both population genetic and epidemiological models often yielded consistent results. For example, both concluded that increasing use of antimalarial drugs promotes the onset of drug resistance [56].

The second contribution of population genetics was made with the introduction of retrospective analysis of antimalarial drug resistance using the genetic footprint generated by selective sweeps [12,44]. Progress in this area is possible due to advances in the coalescent theory that describes the evolution at neutral loci under various scenarios of demography (including complex patterns of population structure) and selection [65]. Under this approach, however, detection of an evolutionary event is possible only when that event changes gene genealogy so it can be observed in the present-day pattern of variation. For example, while an epidemiologists and ecologists will easily agree that a population is growing exponentially by observing it over a short time scale, molecular evolutionary geneticists will not be able to detect this growth unless the process is sustained long enough, in the scale of effective population size and mutation rate, to cause a pattern that can be detected in the sampled genetic variation. If we simply want to predict what will happen in a single population at the end of the transmission season this issue of time scales is discouraging; however, these approaches will be useful to address the dynamic of mutations associated with resistance at a regional scale where several populations with different evolutionary histories need to be compared. The backward-in-time (coalescent) analysis critically depends on the quantity and quality of actual samples from natural populations [65]; nowadays, such sampling has been facilitated by the arrival of high-throughput genotyping methods that can increase the sample size in two dimensions: (i) the number of individuals; and (ii) the portion of the genome being sampled.

An apparent paradox that arises when considering coalescent approximations is that we learn about what has already happened with regard to resistant mutations to drugs such as SP and CQ, rather than actually predicting what is going to happen with the onset of resistance against new drugs such as ACTs. Yet, coalescent approximations can provide valuable information on the dynamic of drug-resistant mutations given the complex demographic history of malarial parasites. Such knowledge can be then applied while studying resistance against new drugs by

defining scenarios with biologically well-defined boundaries. Together, population genetic and population dynamics models could complement each other by considering different level of complexity, time, and spatial scales. The challenge is to build a framework where these approaches can enrich each other.

Perspective

An approach could be to model the dynamic of mutations associated with drug resistance at different spatial and temporal scales with common outputs, such as the standard malaria epidemiology or population metrics. Thus, rather than developing a total model that considers malaria in the world with all its complexity, we could have a group of related models based on the scale of the predictions that need to be achieved but that interact via those common or related sets of parameters. Some of those summary statistics or parameters may allow us to plan for the long-term effect of an intervention strategy (in this case deploying a particular drug at a regional level) while having little to contribute locally. Others, such as those derived or applied to single populations may be very useful for taking decisions in the short term especially if we could consider other factors, such as those related with population structure, negligible.

Such models could be developed by first using a set of preliminary models that allow exploring the sensitivity of the dynamics to changes in such common parameters at a given temporal and/or spatial scale and make a first evaluation based on data [66,67]. Then, such preliminary models will lead to more realistic data-driven models. By considering stochastic processes in models of sufficient complexity, simulated data could be generated and compared with real data. By so doing, models could become real experimental designs where specific scenarios could be rejected due to lack of fit to real data. For example, such an approach may allow us to define what is 'a population' and how it relates with others, providing us with operational units where drug resistance can be managed. Nevertheless, whatever modeling we finally apply to assess how drug resistance emerges and disperses, it would be dramatically improved by a combination of gathering extensive molecular data and well-planned epidemiological investigations.

Origin and spread of resistance to chloroquine and pyramethamine in *P. falciparum*

Chloroquine (CQ)-resistant originated independently in Thailand, in Papua New Guinea and twice in South America [10-12] as indicated by the yellow diamonds numbered 1 to 4 (see Figure I). CQ resistance may also have originated independently also in other regions, such as Cambodia [13] and India [14]. In the case of sulfadoxine-pyrimethamine (SP)-resistance, several alleles of the *dhfr* gene have been associated with resistance to pyrimethamine (PY), one component of SP. Highly resistant alleles, with combinations of three or four mutations, have been reported in Southeast Asia, Melanesia, Africa, and twice in South America [15-20]; they are represented by red triangles numbered 1 to 5. The arrow indicates the introductions of the predominant resistant alleles to both CQ and SP in Africa from Southeast Asia [12,19]. Similar patterns are emerging for those mutations in the gene

encoding *dhps* associated with resistance to sulphadoxine, the other component of SP [17, 20,21]. Comprehensive reviews describing these events are available elsewhere [4,22]. Figure 1a depicts a simplified representation of the evolutionary context where such events took place. Lines in red represent major geographic subdivisions that have been observed with microsatellites [7], mitochondrial genomes [23], and nuclear single nucleotide polymorphisms [24] as indicated by F_{st} values, a measure of genetic differentiation between populations; high F_{st} values indicate high differentiation between populations (>0.2) [7]. Although genetic variation (θ) and recombination (R) varies greatly among populations [7,25]; we are illustrating major emerging patterns using a qualitative scale that goes from ‘-’ or low to ‘+’ or high based on previous reports [7,23-25]. For example, in the case of recombination, areas with ‘+’ indicate high recombination rates where linkage disequilibrium is expected to decay fast with the physical distance among loci and it is seldom observed among loci in different chromosomes [7,25]. Figure 1b, on the other hand, depicts a simplified representation of the ecological and epidemiological context where such events took place. Ovals in green highlight, approximately, areas where there is a high incidence of clinical cases (mesoendemic to hyperendemic [1]). In those areas, drug use is expected to be high. Areas highlighted in blue indicate that malaria is endemic but not with a high number of clinical cases (hypoendemic) so transmission is low and drug use is not expected to be high [26]. ‘MI’ indicates prevalence of multiple infections, or number of patients with infections conformed by more than one parasite lineage. MI is represented by a qualitative scale where ‘-’ is less than 20% of multiple infections [7,17], ‘±’ indicates intermediate levels with more than 20% but less than 40-50% [7], and high (indicated as ‘+’) with more than 50% [7]. It is worth noting that MI varies greatly among populations [7,20]. The prevalence of sub-clinical infections is indicated by ‘A’ in terms of low or ‘-’ (from 0% to $<20\%$ detected by microscopy) and high or ‘+’ with $>20\%$ prevalence of asymptomatic or sub-clinical infections. The challenge is to develop models considering temporal and spatial scales at which selection acts on advantageous mutations in non-equilibrium conditions (Figure 1a: spatially structured populations with different effective population sizes, recombination rates, and a variety of levels of gene flow) and that also consider the epidemiological context that determines drug use and the onset of natural immunity (Figure 1b).

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References

1. Snow RW, et al. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* 2005;434:214–217. [PubMed: 15759000]
2. Laxminarayan R. Act now or later? Economics of malaria resistance. *Am J Trop Med Hyg* 2004;71:187–195. [PubMed: 15331837]
3. Duffy PE, Sibley CH. Are we losing artemisinin combination therapy already? *Lancet* 2005;366:1908–1909. [PubMed: 16325683]
4. Gregson A, Plowe CV. Mechanisms of resistance of malaria parasites to antifolates. *Pharmacol Rev* 2005;57:117–145. [PubMed: 15734729]
5. Roberts MG. The pluses and minuses of R_0 . *J R Soc Interface* 2007;4:949–961. [PubMed: 17490942]
6. Koella JC, Antia R. Epidemiological models for the spread of anti-malarial resistance. *Malar J* 2003;2:3. [PubMed: 12643812]

7. Anderson TJ, et al. Microsatellite markers reveal a spectrum of population structures in the malaria parasite *Plasmodium falciparum*. *Mol Biol Evol* 2000;17:1467–1482. [PubMed: 11018154]
8. Hastings IM. The origins of antimalarial drug resistance. *Trends Parasitol* 2004;20:512–518. [PubMed: 15471702]
9. Charlesworth B. Fundamental concepts in genetics: effective population size and patterns of molecular evolution and variation. *Nat Rev Genet* 2009;10:195–205. [PubMed: 19204717]
10. Chen N, et al. Origin and dissemination of chloroquine-resistant *Plasmodium falciparum* with mutant pfcrt alleles in the Philippines. *Antimicrob Agents Chemother* 2005;49:2102–2105. [PubMed: 15855538]
11. Mehlotra RK, et al. Evolution of a unique *Plasmodium falciparum* chloroquine-resistance phenotype in association with pfcrt polymorphism in Papua New Guinea and South America. *Proc Natl Acad Sci U S A* 2001;98:12689–12694. [PubMed: 11675500]
12. Wootton JC, et al. Genetic diversity and chloroquine selective sweeps in *Plasmodium falciparum*. *Nature* 2002;418:320–323. [PubMed: 12124623]
13. Lim P, et al. pfcrt polymorphism and chloroquine resistance in *Plasmodium falciparum* strains isolated in Cambodia. *Antimicrob Agents Chemother* 2003;47:87–94. [PubMed: 12499174]
14. Vinayak S, et al. Wide variation in microsatellite sequences within each Pfcrt mutant haplotype. *Mol Biochem Parasitol* 2006;147:101–108. [PubMed: 16516311]
15. Cortese JF, et al. Origin and dissemination of *Plasmodium falciparum* drug-resistance mutations in South America. *J Infect Dis* 2002;186:999–1006. [PubMed: 12232841]
16. McCollum AM, et al. Antifolate resistance in *Plasmodium falciparum*: multiple origins and identification of novel dhfr alleles. *J Infect Dis* 2006;194:189–197. [PubMed: 16779725]
17. McCollum AM, et al. Common Origin and Fixation of *Plasmodium falciparum* dhfr and dhps Mutations Associated with Sulfadoxine-Pyrimethamine Resistance in a Low-Transmission Area in South America. *Antimicrob Agents Chemother* 2007;51:2085–2091. [PubMed: 17283199]
18. Roper C, et al. Antifolate antimalarial resistance in southeast Africa: a population-based analysis. *Lancet* 2003;361:1174–1181. [PubMed: 12686039]
19. Roper C, et al. Intercontinental spread of pyrimethamine-resistant malaria. *Science* 2004;305:1124. [PubMed: 15326348]
20. McCollum AM, et al. Hitchhiking and selective sweeps of *Plasmodium falciparum* sulfadoxine and pyrimethamine resistance alleles in a population from central Africa. *Antimicrob Agents Chemother* 2008;52:4089–4097. [PubMed: 18765692]
21. Pearce RJ, et al. Multiple origins and regional dispersal of resistant dhps in African *Plasmodium falciparum* malaria. *PLoS Med* 2009;6:e1000055. [PubMed: 19365539]
22. Hayton K, Su XZ. Drug resistance and genetic mapping in *Plasmodium falciparum*. *Curr Genet* 2008;54:223–239. [PubMed: 18802698]
23. Joy DA, et al. Early origin and recent expansion of *Plasmodium falciparum*. *Science* 2003;300:318–321. [PubMed: 12690197]
24. Volkman SK, et al. A genome-wide map of diversity in *Plasmodium falciparum*. *Nat Genet* 2007;39:113–119. [PubMed: 17159979]
25. Mu J, et al. Recombination hotspots and population structure in *Plasmodium falciparum*. *PLoS Biol* 2005;3:1734–1741.
26. Guerra CA, et al. The limits and intensity of *Plasmodium falciparum* transmission: implications for malaria control and elimination worldwide. *PLoS Med* 2008;5:e38. [PubMed: 18303939]
27. Nair S, et al. Recurrent gene amplification and soft selective sweeps during evolution of multidrug resistance in malaria parasites. *Mol Biol Evol* 2007;24:562–573. [PubMed: 17124182]
28. Mita T, et al. Independent evolution of pyrimethamine resistance in *Plasmodium falciparum* isolates in Melanesia. *Antimicrob Agents Chemother* 2007;51:1071–1077. [PubMed: 17210777]
29. Nair S, et al. A selective sweep driven by pyrimethamine treatment in southeast asian malaria parasites. *Mol Biol Evol* 2003;20:1526–1536. [PubMed: 12832643]
30. Pearce R, et al. Reduced Variation Around Drug-Resistant dhfr Alleles in African *Plasmodium falciparum*. *Mol Biol Evol* 2005;22:1834–1844. [PubMed: 15917494]

31. Laufer MK, et al. Return of chloroquine antimalarial efficacy in Malawi. *N Engl J Med* 2006;355:1959–1966. [PubMed: 17093247]
32. Mita T, et al. Expansion of wild-type allele rather than back mutation in *pfcr* explains the recent recovery of chloroquine sensitivity of *Plasmodium falciparum* in Malawi. *Mol Biochem Parasitol* 2004;135:159–163. [PubMed: 15287598]
33. Zhou Z, et al. Decline in sulfadoxine-pyrimethamine-resistant alleles after change in drug policy in the Amazon region of Peru. *Antimicrob Agents Chemother* 2008;52:739–741. [PubMed: 18025120]
34. Takahashi KR. Evolution of coadaptation in a subdivided population. *Genetics* 2007;176:501–511. [PubMed: 17409093]
35. Whitlock MC. Fixation probability and time in subdivided populations. *Genetics* 2003;164:767–779. [PubMed: 12807795]
36. Otto SP, Whitlock MC. The probability of fixation in populations of changing size. *Genetics* 1997;146:723–733. [PubMed: 9178020]
37. Hadany L. Adaptive peak shifts in a heterogeneous environment. *Theor Pop Biol* 2003;63:41–51. [PubMed: 12464494]
38. Babiker HA, et al. Drug resistant *Plasmodium falciparum* in an area of seasonal transmission. *Acta Trop* 2005;94:260–268. [PubMed: 15857801]
39. Ord R, et al. Seasonal carriage of *pfcr* and *pfmdr1* alleles in Gambian *Plasmodium falciparum* imply reduced fitness of chloroquine-resistant parasites. *J Infect Dis* 2007;196:1613–1619. [PubMed: 18008244]
40. Klein EY, et al. Clinically immune hosts as a refuge for drug-sensitive malaria parasites. *Malar J* 2008;7:67. [PubMed: 18439283]
41. Bray PG, et al. Defining the role of *PfCRT* in *Plasmodium falciparum* chloroquine resistance. *Mol Microbiol* 2005;56:323–333. [PubMed: 15813727]
42. Sirawaraporn W, et al. Antifolate-resistant mutants of *Plasmodium falciparum* dihydrofolate reductase. *Proc Natl Acad Sci U S A* 1997;94:1124–1129. [PubMed: 9037017]
43. Anderson TJ, Roper C. The origins and spread of antimalarial drug resistance: lessons for policy makers. *Acta Trop* 2005;94:269–280. [PubMed: 15878153]
44. Nash D, et al. Selection strength and hitchhiking around two anti-malarial resistance genes. *Proc Biol Sci* 2005;272:1153–1161. [PubMed: 16024377]
45. Smith JM, Haigh J. The hitch-hiking effect of a favorable gene. *Genet Res* 1974;23:23–35. [PubMed: 4407212]
46. Barton NH. Genetic hitchhiking. *Philos Trans Biol Sci* 2000;355:1553–1562.
47. Kim Y, Stephan W. Detecting a local signature of genetic hitchhiking along a recombining chromosome. *Genetics* 2002;160:765–777. [PubMed: 11861577]
48. Wiehe T. The effect of selective sweeps on the variance of the allele distribution of a linked multiallele locus: hitchhiking of microsatellites. *Theor Popul Biol* 1998;53:272–283. [PubMed: 9679322]
49. Kim Y, Nielsen R. Linkage disequilibrium as a signature of selective sweeps. *Genetics* 2004;167:1513–1524. [PubMed: 15280259]
50. McVean G. The structure of linkage disequilibrium around a selective sweep. *Genetics* 2007;175:1395–1406. [PubMed: 17194788]
51. Fay JC, Wu CI. Hitchhiking under positive Darwinian selection. *Genetics* 2000;155:1405–1413. [PubMed: 10880498]
52. Nielsen R, et al. Genomic scans for selective sweeps using SNP data. *Genome Res* 2005;15:1566–1575. [PubMed: 16251466]
53. Begun DJ, Aquadro CF. Levels naturally occurring DNA polymorphism correlate with recombination rates in *D. melanogaster*. *Nature* 1992;356:519–520. [PubMed: 1560824]
54. Barton NH. Linkage and the limits to natural selection. *Genetics* 1995;140:821–841. [PubMed: 7498757]
55. Presgraves DC. Recombination enhances protein adaptation in *Drosophila melanogaster*. *Current Biology* 2005;15:1651–1656. [PubMed: 16169487]
56. Mackinnon MJ. Drug resistance models for malaria. *Acta Trop* 2005;94:207–217. [PubMed: 15894181]

57. Bonnet S, et al. Estimation of malaria transmission from humans to mosquitoes in two neighbouring villages in south Cameroon: evaluation and comparison of several indices. *Trans R Soc Trop Med Hyg* 2003;97:53–59. [PubMed: 12886806]
58. Githeko AK, et al. The reservoir of *Plasmodium falciparum* malaria in a holoendemic area of western Kenya. *Trans R Soc Trop Med Hyg* 1992;86:355–358. [PubMed: 1359683]
59. Hastings IM, Watkins WM. Intensity of malaria transmission and the evolution of drug resistance. *Acta Trop* 2005;94:218–229. [PubMed: 15847846]
60. Pongtavornpinyo W, et al. Spread of anti-malarial drug resistance: mathematical model with implications for ACT drug policies. *Malar J* 2008;7:229. [PubMed: 18976503]
61. Ross, R. *The Prevention of Malaria*. 1st. John Murray; 1910.
62. MacDonald, G. *The Epidemiology and Control of Malaria*. Oxford University Press; 1957.
63. Bonhoeffer S, et al. Evaluating treatment protocols to prevent antibiotic resistance. *Proc Natl Acad Sci U S A* 1997;94:12106–12111. [PubMed: 9342370]
64. Hastings IM. Complex dynamics and stability of resistance to antimalarial drugs. *Parasitol* 2006;132:615–624.
65. Rosenberg NA, Nordborg M. Genealogical trees, coalescent theory and the analysis of genetic polymorphisms. *Nat Rev Genet* 2002;3:380–390. [PubMed: 11988763]
66. Smith DL, et al. The entomological inoculation rate and *Plasmodium falciparum* infection in African children. *Nature* 2005;438:492–495. [PubMed: 16306991]
67. Smith DL, et al. Revisiting the basic reproductive number for malaria and its implications for malaria control. *PLoS Biol* 2007;5:e42. [PubMed: 17311470]

Glossary of terms

Coalescent Theory	All alleles in a sample are descendants from a common ancestor. Therefore, going backward in time, ancestral lineages of alleles sequentially merge (coalesce) to form a single gene genealogy. By modeling this stochastic process, a distribution of genealogies emerges, allowing the estimation of parameters of interest from a sample of alleles, such as the time to the most recent common ancestor or the effective population size.
Effective Population Size (N_e)	Is the size of an ideal population that has the same level of variation, determined by random genetic drift, as an actual population does.
Fitness	This is the expected number of offspring that each genotype produces in a given environment. Most population genetic models use a standardized measure or relative fitness that goes from 0 to 1. It is calculated by dividing the absolute fitness of each genotype by the absolute fitness of the fittest genotype; thus, the fittest genotype has a relative fitness of one.
Genetic drift	Random changes of the allele frequencies in the population due to the sampling of gametes from the parental gene pool to make zygotes. The higher the number of individuals contributing to the next generation, the lower the fluctuations in the allele frequencies by chance.
Genetic equilibrium	is the state at which the allele distribution and the frequencies of mutations are constant over time.
Gene flow	The exchange of alleles between populations or subpopulations. The lower the gene flow, the higher the genetic differentiation between populations or subpopulations. It is how migration is assessed in most population genetic models.

Genetic hitchhiking	is the process by which neutral mutations increase in frequency by virtue of being in the neighborhood of (or linked to) an advantageous mutation that is undergoing a selective sweep.
Linkage disequilibrium (LD)	Alleles at different loci have a tendency to be inherited together. It could be the result of proximity in the same chromosome, non-random mating such as inbreeding, natural selection, among other factors.
Population structure	Individuals are distributed into groups or subpopulations where mating is more likely to take place and/or individuals are placed under the same selective pressure; thus, the allele frequencies among subpopulations become different. The extent of such differentiation depends on the level of gene flow. Such structure can be the result of, for example, geographic isolation, usually referred to as geographic structure.
R_0	The average number of secondary cases a typical infectious individual will cause in a susceptible population.
Selection coefficient (s)	it is a measure of the relative fitness of a genotype, it ranges $0 \leq s \leq 1$.
Selective sweep	is when an advantageous mutation increases in frequency driven by strong positive natural selection eliminating neutral variation (that does not affect fitness) among neighboring (linked) sites.