Plasticity in parasite phenotypes: evolutionary and ecological implications for disease

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Preventing disease is a major goal of applied bioscience and explaining variation in the harm caused by parasites, and their infectiousness, are major goals of evolutionary biology. The emerging field of evolutionary medicine integrates these two ambitions to inform the development of control strategies that retard or withstand unfavorable parasite evolution. However, as parasites live in hostile and changeable environments – the bodies of other organisms – the success of integrating evolutionary biology with medicine requires a better understanding of how natural selection has solved the problems parasites face. There is increasing appreciation that natural selection shapes parasite strategies to survive in the host and transmit between hosts through facultative (plastic) shifts in parasite traits expressed during infections and in different hosts. This article describes how integrating parasite plasticity into biomedical thinking is central to explaining disease outcomes and transmission patterns, as well as predicting the success of control measures.

Natural selection is clearly important for biomedicine (i.e., it is the process that produces drug-resistant parasites). Consequently, evolutionary biology ought to be better integrated into the development and implementation of disease control measures [1,2]. Evolutionary and ecological frameworks provide testable hypotheses to explain the strategies that parasites have evolved to maximize their success, can explain disease outcomes, and inform the development of sustainable control measures. Research in evolutionary medicine asks how and why parasites 'do what they do' and 'are the way they are'. For example, why is there so much variation in the severity of infections with the same parasite species? And, to what extent is this explained by variation in parasite traits, host responses or complex interactions between hosts and parasites? This variation is the raw material and product of evolution; therefore, explaining its existence is key to understanding how medical interventions can drive evolutionary changes [3].

An evolutionary and ecological perspective casts this variation in another light as well: it recognizes parasites as responsive organisms that can use cues from their in-host environment to quickly alter their traits and behaviors in ways that maximize fitness in changing circumstances [4,5]. This approach can overturn assumptions about parasite biology, reveal unexpected sophistication in their strategies, and identify clinically positive or negative evolutionary consequences of interventions.

Here, we review experiments suggesting that parasites have evolved to employ a variety of flexible (plastic) strategies to survive in the host and transmit between hosts, and explain why parasite traits are shaped by ecological interactions between parasites and their hosts or vectors. Parasite traits underpinning in-host survival and between-host transmission are determinants of the virulence and infectiousness phenotypes that natural selection acts on, and so these traits shape disease severity, epidemiology and the ability of parasites to counter control measures. We propose that a better understanding of infectious disease requires integrating traditional approaches for studying parasite biology with evolutionary and ecological frameworks. For example, cell and molecular parasitologists rarely quantify within-species variation in the genes and molecules underpinning physiological processes, and population studies of disease tend to focus on the patterns of transmission, avoiding the details of processes that occur during infections, which ultimately give rise to these patterns of transmission. Evolutionary ecology can bridge this divide by providing context to integrate understanding of cell and molecular processes underpinning disease with patterns observed at the levels of populations and species (Figure 1). We focus on malaria (*Plasmodium*) parasites for which there has been
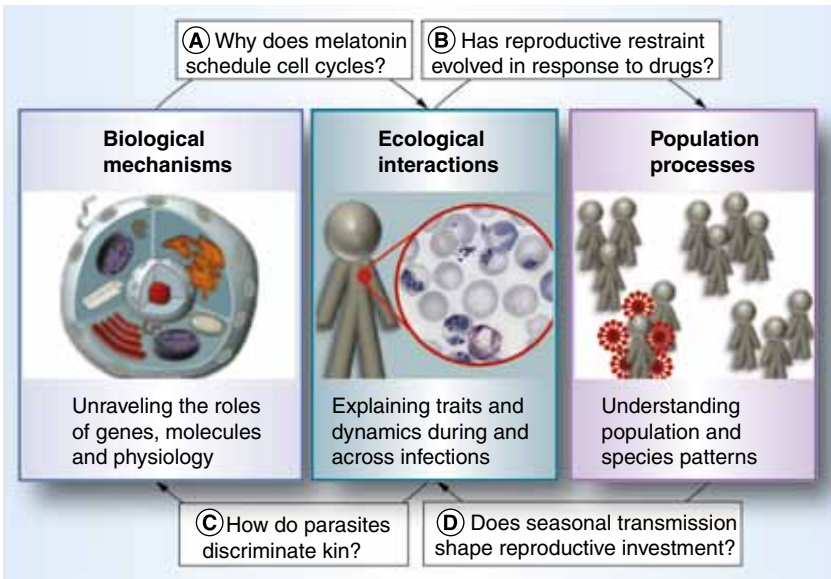
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Figure 1. Ecological interactions. Ecological interactions between parasites, and between parasites and hosts, shape parasite traits. These interactions occur at an important intermediate level of biological organization that bridges between scales of disease that are traditionally studied separately and with different approaches (i.e., cell biology and population biology). This is illustrated above, for example: **(A)** information about the molecular mechanisms underlying parasite traits is key to understanding the constraints on trait evolution; **(B)** the traits that parasites exhibit during infection underpin the virulence and transmission phenotypes that are exposed to natural selection; **(C)** adaptive plasticity in traits reveals mechanisms yet to be uncovered, which may be targets for interventions; and **(D)** identifying processes and parameters that are important at the host population level can shed light on what parasites are doing within infections.

considerable integration of disciplines to ask questions about disease ecology and evolution. We introduce the central concepts from evolutionary and ecological theory, summarize the current state of affairs, and outline the key future challenges and how they can be met.

Plastic parasites

Parasites experience rapid and extensive variation in environmental conditions inside hosts and vectors. There is increasing evidence for flexibility in parasite traits that underpin in-host replication and between-host transmission (spanning from immune evasion traits to investment in transmissible forms) in response to variation in their environment (e.g., resource availability, in-host competition and drug treatment) throughout infections and in different hosts [5]. In evolutionary ecology, this flexibility in traits is referred to as 'phenotypic plasticity': the ability of a genotype to produce different phenotypes as the environment changes [6]. Phenotypic plasticity is a ubiquitous evolutionary solution to the challenges of life in a changing environment as it enables organisms to maximize fitness by altering their phenotype

according to their circumstances, through mechanisms such as differential gene expression. Whilst the molecular signaling pathways that many parasites use to respond to environmental change are well characterized, the fact that plasticity can produce qualitative and strategic (i.e., adaptive) changes to phenotypes has been overlooked.

Understanding plasticity in parasite traits is important as it has the potential to impact the severity, incidence and transmission of disease, as well as the success of control measures. For example:

- As there is renewed interest in developing transmission-blocking interventions, understanding plasticity in transmission strategies is central to predicting the consequences of such control measures [7];

- Identifying the cues that stimulate changes in parasite traits may provide targets for interventions that manipulate parasites into making decisions that reduce their fitness (reducing virulence and/or transmission) [8];

- Moreover, when the mechanisms that allow for plasticity in a trait are correlated with other important life functions, parasite counter-evolution can be constrained, potentially making such interventions 'evolution proof' [9];

- Within-host infection dynamics are usually considered to be under the control of host regulatory mechanisms (e.g., immune responses and/or resources, see [10] for an example); therefore, asking how parasites respond to changes in the environment can overturn assumptions about their role in shaping infection dynamics and clinical outcomes (whether or not plasticity maximizes parasite fitness);

- It is often assumed that plasticity only generates marginal changes to phenotypes. However, as parasite phenotypes are a product of several plastic traits and since multiple environmental factors change simultaneously, a wide range of phenotypes are possible. Furthermore, when different genotypes respond to the environment in different ways (genotype-by-environment interactions), this results in considerable phenotypic variation for natural selection to act on [11];

- It is widely recognised that environmental change, including drugs/vaccines, can drive the evolution of parasite phenotypes (e.g., through mutation or recombination), but

plasticity can similarly produce new pheno-types or change parasite traits and on even shorter timescales.

Parasite traits

Malaria (*Plasmodium* spp.) and related parasites are some of the most serious pathogens affecting humans, livestock, companion animals and wildlife. In-host replication and between-host transmission are achieved by different specialized stages (**Figure 2**). Cycles of asexual replication inside red blood cells (RBCs) enable parasites to exploit the host in order to establish and maintain infections, but to infect the mosquito vector and transmit to new hosts, specialized sexual stages (gametocytes) that do not replicate in the host are produced. When taken up by a vector, male and female gametocytes rapidly differentiate into gametes, fertilization occurs in the blood meal and the offspring (ookinetes) traverse the midgut where they form cysts that produce stages infective to new hosts. The density of gametocytes and their sex ratio determine the reproductive success and, therefore, fitness (genetic contribution to subsequent generations) of malaria parasites. Traits underpinning in-host replication influence the ability to exploit host resources and survive adverse conditions. In the following sections, we focus on what is known about plasticity in traits involved in between-host transmission and in-host replication as they have important consequences epidemiologically (e.g., force of infection) and clinically (e.g., disease severity).

State of affairs: reproductive traits

Evolutionary ecology provides a framework for predicting and explaining how organisms schedule investment in reproduction throughout their lives [12,13]. The requirement of different stages for in-host survival and between-host transmission means that malaria parasites face the same allocation trade-offs common to all sexually reproducing organisms [13]: resources must be divided between replicating asexually (growth/maintenance) and producing gametocytes (reproduction), and resources devoted to gametocytes must be divided between males and females (sex ratio). In multicellular taxa, individual organisms are easily identifiable as the target of natural selection (i.e., selection maximizes the fitness of individuals). For single-celled parasites, each group of clonally related parasites within an infection is the comparable target [14]. Therefore, when infections consist of a single genotype, selection maximizes fitness over the lifetime of the infection.

Evolutionary theory predicts that investment in gametocytes affects fitness by influencing virulence and the rate and duration of transmission [15]. A parasite genotype that invests heavily in gametocytes early in infections may not produce sufficient densities of asexual parasites to maintain an infection (e.g., in the face of immune responses); the window of opportunity for transmission may therefore be short. Conversely underinvestment in gametocytes reduces the rate of transmission and could also curtail the duration, if infections are so virulent (from high investment in host-damaging asexual parasite forms) that the host dies.

Most work considering phenotypic plasticity in malaria parasites has focused on reproductive traits (investment into gametocytes and their sex ratio). These traits are conceptually straightforward to study because the trade-offs are clear and the relative investment in different stages is easy to quantify. Work using the rodent malaria parasite model, *Plasmodium chabaudi,* and the human malaria parasite, *Plasmodium falciparum*, in culture suggests that parasites adaptively adjust their investment into sexual stages and their sex ratio in response to changes in the in-host environment experienced during infections [16–22]. For example, parasites adjust the ratio of male to female
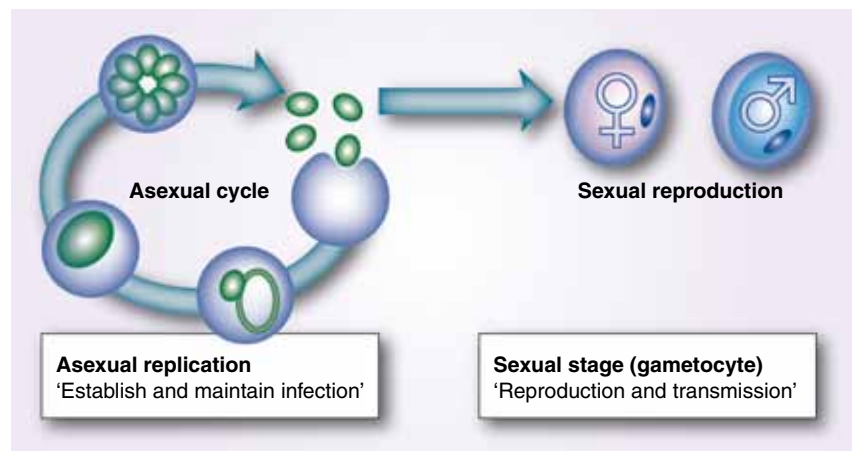

Figure 2. Malaria blood stages and traits. Cycles of asexual replication inside red blood cells enable parasites to exploit the host to establish and maintain infections, but to infect the mosquito vector and transmit between hosts, specialized sexual stages (gametocytes) are produced. The density of gametocytes and their sex ratio (proportion that are male) influence the success of establishing in mosquito vectors and, therefore, between-host transmission. Considerable variation is observed in the production of sexual stages and their sex ratio during infections and in different hosts. Understanding how parasite and host factors contribute to this variation is central to explaining patterns of disease transmission and predicting plastic (and evolutionary) responses to interventions. Data suggest parasites adjust the level of investment in reproduction in response to changes in the in-host environment experienced during infections in ways that maximize their fitness. While such variation is also expected for traits underpinning asexual replication rate, few empirical studies have sought to quantify and understand any variation in these traits in response to in-host environmental changes.

gametocytes according to the genetic diversity of infections, as this determines their inbreeding rate [20]. Briefly, because each male sexual stage is capable of fertilizing several females, the evolutionarily optimal sex ratio shifts from highly female-biased to equality as the inbreeding rate decreases from complete inbreeding to outcrossing [23]. When parasites experience competitive suppression, RBC resource limitation, or low doses of antimalarial drugs they reduce investment in gametocytes (i.e., adopt 'reproductive restraint') [19,24]. Other studies have demonstrated that high drug doses, which severely reduce survival probability, induce investment in gametocytes at the expense of continued in-host replication (i.e., 'terminal investment') [16,17]. These apparently contradictory strategies can be understood by considering the severity of the 'stress' imposed by changes in the in-host environment [25]. Under low stress (e.g., early in infections of naive hosts) parasites can afford gametocytes, but as conditions deteriorate (e.g., in-host competition, anemia) parasites must employ 'reproductive restraint' and reduce gametocyte investment to ensure in-host survival and the potential for future transmission. Under extreme stress, when the death rate exceeds the potential for replication (e.g., radical drug treatment) parasites should switch to a 'terminal investment': by diverting all resources to gametocytes they maximize short-term transmission. Terminal investment is also predicted when organisms face circumstances likely to be fatal (e.g., host death). However, only a small proportion of malaria infections are fatal (due to drugs/partial immunity), so selection for terminal investment is more likely to be driven by in-host factors that threaten to clear parasites.

Using frameworks from evolutionary ecology to explain parasite strategies requires better integration with knowledge of the mechanistic processes governing in-host and between-host processes [5]. At the in-host level, it remains unclear which cues parasites are using to alter their reproductive strategies and, therefore, how fine-tuned these strategies can be to environmental variation. At the between-host level, data are required to test whether different reproductive strategies actually map to the rate and duration of transmission as predicted by evolutionary theory (i.e., does reproductive restraint under stress enhance fitness by prolonging the duration of transmission? In lethal situations, does terminal investment pay off by increasing short-term transmission?) This link is important for establishing whether plasticity in reproductive decisions is adaptive. Ultimately, in-host and between-host processes also need to

be integrated to understand and predict parasite evolution: conditions experienced within the host influence reproductive strategies; these strategies play a major role in determining the success of transmission to vectors, as well as having downstream effects on the prevalence and intensity of infection in the vector, which ultimately govern transmission to new hosts. Recognizing the role that plasticity plays in shaping all of these processes, and quantifying its importance, is the first step towards this integration.

State of affairs: replication traits

Whilst progress has been made in understanding plasticity in traits that shape transmission from hosts to vectors, and how investment in transmission (gametocytes) trades off against investment in asexual replication, few empirical studies have sought to understand how conditions in the host influence variation in traits involved in asexual replication. These traits include: the duration, schedule and synchronicity of cell cycles; the number of progeny produced per asexually replicating parasite; preference for host blood cells of different ages; rate of switching molecules recognized by host immunity; and interactions with host cells (e.g., sequestration). Little is known about plasticity and trade-offs governing the traits underpinning replication and how this shapes strategies for in-host survival. Evolutionary theory for parasites has also tended to ignore these traits, focusing instead on virulence. This is problematic because a change in virulence is achieved by changes in underlying traits [26]. As the traits underpinning virulence are likely to be linked by genetic correlations (e.g., different traits are shaped by the same genes) and/or resource allocation trade-offs [27], the nature of these interactions is central to understanding and predicting virulence evolution. For example, faster replication could be achieved by increasing the number of progeny per parasite (burst size), but if there is a constraint in which increasing burst size requires a longer cell cycle duration then the extent of plastic adjustment may be limited. Explaining variation in host exploitation strategies is also central to understanding the transmission consequences of in-host processes: if parasites have evolved to invest in in-host survival when stressed, it is important to know how (in addition to reproductive restraint) they achieve this.

Experiments with *P. chabaudi in vivo* and *P. falciparum in vitro* suggest that there is plasticity and genetic variation for replication traits, with considerable effects on virulence and

transmission [5,28,29]. For example, *P. falciparum* cell cycle duration diverges from 48 h, and synchronicity is lost in culture [30]. Perturbed rodent malaria parasite cell cycles reschedule during an infection to match the host circadian rhythm [31], and we have recently demonstrated that non-'matched' parasites pay a twofold cost in the production of sexual and asexual stages [32]. Whether the cell cycle is passively scheduled by host factors with a circadian basis [33], or parasites are adaptively able to adjust their timing, is a key open question. Hosts can only be partially responsible: parasites respond to the hosts' circadian release of melatonin [34], but synchronous and asynchronous malaria parasite species are able to maintain their schedules in the same host environment [35]. Parasites may actively adjust their schedules to maximize exploitation of a circadian resource (e.g., when RBCs enter the circulation [36]), or to avoid exposing a vulnerable developmental stage to immune factors with a circadian rhythm (e.g., TNF-α) [37]. Ring stages have been implicated in plasticity in cell cycle duration, and the ability to reversibly enter a quiescent state during exposure to drugs (especially artemisinin) is implicated in drug tolerance [38]. Understanding plasticity in cell cycles, therefore, has applied importance.

Our work has recently suggested that parasites adjust yet another component of replication in response to the in-host environment. When the availability and age structure of host RBCs was experimentally altered, we found that parasite burst sizes increased [39]. Again, whilst parasites are plastically adjusting burst sizes, it is unclear whether this response is adaptive (e.g., if increasing burst size when resources are scarce ensures enough progeny parasites find a RBC to infect). Testing the fitness consequences of variation in burst size is necessary to bring the understanding of replication strategies in line with that of reproductive strategies. Integrating mechanistic in-host models and experimental tests is an efficient way of progressing knowledge of how replication traits, and plasticity thereof, change across infections and over evolutionary time.

Evolutionary consequences

Plasticity and evolution (change in gene frequencies) are not mutually exclusive (i.e., mechanisms enabling plasticity can evolve, see [42] for an example) and plasticity has the potential to constrain or facilitate evolution when the environment changes. In a novel environment, plasticity can facilitate evolution by providing more time/opportunities for beneficial mutations to arise because population survival is enhanced. By contrast, when plasticity buffers organisms against the loss of fitness in a novel environment, the strength of selection imposed by environmental change is reduced, so evolution is constrained (see [43] for an example). Understanding when plasticity will facilitate or constrain evolutionary change requires quantifying the costs and limits of plasticity, the strength of selection, and the mutation rate. Although this is no small task [44], work on the evolutionary ecology of parasites begins to shed light on the first two of these factors by studying the range and relative fitness of parasite phenotypes exhibited under different environmental conditions. Far from an academic exercise, knowing when plasticity would facilitate or constrain evolution has implications for disease control.

Consider the following: recent work has warned that reproduction and replication traits can buffer parasites from the impact of drugs. In *in vitro* studies, multiple field isolates of drug-sensitive *P. falciparum* adopt reproductive restraint in the face of drug pressure, whereas drug-resistant isolates invest in gametocytes at a higher rate and do not adjust investment in control compared to drug-treated cultures [19]. Rodent malaria experiments show that faster replicating (more virulent) parasite genotypes are better able to survive in, and disproportionately contribute to transmission from, drug-treated infections [45]. If plasticity provides tolerance to drugs, then selection for resistance traits that require genetic changes (e.g., efflux pumps and alternative metabolic pathways) is reduced because plasticity reduces the sensitivity of parasites to drugs. Whilst this might seem advantageous from the perspective of slowing the emergence and spread of fixed resistance traits, the downside is that plasticity reduces the efficacy of the drug on a very short timescale. Just how short this timescale is, how much the efficacy of the drug is reduced and which parasite traits are involved will determine whether tolerance is a more favorable outcome epidemiologically (e.g., total number of infections) or clinically (e.g., severity of disease) than if parasites evolved fixed drug resistance traits.

Future perspective

The body of tools and insight generated from evolutionary and ecological research on malaria parasites suggests that there is great potential for

applied impact. However, there are major challenges to overcome before informed evolutionary and ecological thinking about parasite plasticity can be effectively translated into medicine and public health. First, much of the knowledge generated thus far has used rodent models or human parasites in culture. These laboratory systems have provided invaluable insight, from the molecular biology of malaria parasites to epidemiology. However, attention to species-specific ecological details is required when plasticity in phenotypes is a result of changing in-host conditions. In other words, subtle differences between rodent and human hosts or between *in vitro* and *in vivo* conditions may differentially impact on phenotypes. Translating findings from laboratory systems to natural infections requires regular sampling of infections, during and after treatment, to measure multiple parasite and host parameters [46]. This will soon be feasible for reproductive traits because tools are increasingly becoming available and the hypotheses are clear. By contrast, directing studies of replication traits requires work on laboratory systems to better define the questions.

We contend that given the considerable amount of plasticity observed in experimental infections – where host genetics, age, sex and a suite of other environmental variables are highly controlled (and in culture where the environment is even less changeable or complex) – there is every reason to expect that plasticity in natural infections will be an important aspect of parasite strategies for success, including evasion of interventions. For example, there is renewed interest in developing transmission-blocking interventions that prevent parasites from mating in the mosquito blood meal [47]. However, given that plasticity observed in sex ratio spans most of the biological range possible for this trait [20,22,48] parasites can readily adjust the production of males and females during infections to partially compensate for interventions with sex-specific effects on fertility [7,49].

Conclusion

Natural selection shapes the severity and infectiousness of parasites and some of the key obstacles for controlling disease are evolutionary problems. Thus, the solutions to these problems lie, at least in part, in a better understanding of parasite evolution. For example, given that the drugs pipeline is running dry and vaccines are not available for many diseases, strategies based on sound evolutionary ecological foundations that make better use of available drugs are necessary [50]. By integrating knowledge at different biological scales – from cells, to ecology, to epidemiology – it may be possible to develop public health tools for manipulating plasticity in parasite traits and behaviors through interventions that 'trick' parasites into adopting strategies that are of clinical or, epidemiological benefit. For example, in controlled (vector-free) hospital

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Executive summary

Evolutionary concepts & parasite traits

- Parasites, like all organisms, interact with their environment in dynamic ways; their traits shape, and are shaped by, the in-host environment.
- Evolutionary ecology offers an interdisciplinary perspective for studying parasite strategies, and decades of research using multicellular taxa validate the utility of this approach.
- Evolutionary and ecological research will define the roles of parasites, hosts and their interactions in the clinical outcomes and transmission of disease.

Transmission & survival traits

- Parasite reproductive strategies appear to be highly sophisticated: parasites are able to fine tune responses according to the severity of a given stress, and evolutionary and ecological theory predicts that these responses maximize fitness.
- Despite a lack of data, there is no reason to expect that adaptive plasticity does not also exist for replication traits. Few studies have yet to explicitly address this question.
- Plasticity enables parasites to adjust a trait across much of the biologically possible range when circumstances change, without requiring evolutionary change.

Consequences & future perspective

- Plasticity means that traits are not static and so measurements need to be made within the context of the environment experienced during infections and across hosts/vectors.
- Quantifying the fitness consequences of plasticity is required to establish how much trait variation is adaptive. Identifying a rigorous approach for doing this remains a major challenge in evolutionary ecology more broadly.
- A better understanding of the causes and consequences of plasticity is required to make the best use of available interventions and may reveal how to 'trick' parasites into making suboptimal decisions.

conditions, inducing all parasites to commit to nonreplicating transmission stages would reduce the virulence of infections and could also produce an effective transmission-blocking immune response that acts against future infections, or inducing parasites to permanently remain quiescent would reduce pathology and could also boost immunity. The explanatory and predictive powers of evolutionary and ecological theories have been validated with decades of research on multicellular taxa. Applying this approach and body of tools to study parasites and the diseases they cause offers a promising and novel way to integrate the role of plasticity in parasite traits with biomedicine.

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