

The many faces of epidemiology: evolutionary epidemiology

As muitas faces da epidemiologia: epidemiologia evolucionária

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Abstract *We review important issues revealed by the application of the evolutionary theory to epidemiological problems. The scope is restricted to infectious diseases and the evolution of virulence as a consequence of public health strategies to control transmission. We focus on the discussion about the possibility of virulence management and explore current scenarios in which recent advances in molecular biology and genetics offer new tools to monitor and change diversity among pathogens, vertebrate and invertebrate hosts. We stress the need to integrate the analytical framework of epidemiology into population genetics and evolutionary theory. We anticipate as an outcome of this process the development of study designs and analytical tools to predict the evolutionary implications of control measures in the population and surveillance mechanisms to continuously monitor the changes in pathogen virulence patterns. Communication among modelers, epidemiologists and molecular biologists is essential in order to design model-driven field trials and to develop data-driven analytical tools leading to conclusive findings that can inform the public health oriented decision making process.*

Key words *Evolutionary epidemiology, Infectious diseases, Virulence management, Molecular biology, Population genetics*

Resumo *Apresentamos os principais conceitos relacionados à aplicação da teoria evolutiva a problemas epidemiológicos. Limitamo-nos às doenças infecciosas e à evolução da virulência como consequência das estratégias de controle da transmissão em saúde pública. Nosso foco é voltado à discussão sobre a possibilidade de controle da virulência e exploramos possíveis cenários atuais em que os avanços recentes em biologia molecular e genética oferecem novas ferramentas de controle e monitoramento de variações na diversidade em patógenos e hospedeiros. Chamamos a atenção para a necessidade de integrar a estrutura analítica da epidemiologia com a genética de populações e a teoria evolutiva. Seguindo a tradição epidemiológica, antecipamos como resultado deste processo o desenvolvimento de desenhos de estudos e ferramentas analíticas de predição das implicações evolutivas das medidas de controle usadas em populações e mecanismos de vigilância que permitam o monitoramento contínuo de mudanças nos padrões de virulência de patógenos. A comunicação entre modeladores, epidemiologistas e biólogos moleculares torna-se essencial ao desenho de ensaios de campo motivados por dados empíricos e ao desenvolvimento de ferramentas analíticas que possam informar o processo de decisão orientado aos problemas em saúde pública.*

Palavras-chave *Epidemiologia evolutiva, Doenças infecciosas, Controle da virulência, Biologia molecular, Genética de populações*

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Introduction

Evolutionary concepts have been influencing the thinking in the health sciences for quite some time now¹. The contributions range from noninfectious and degenerative diseases² to infectious diseases and public health³ and provide insights into fields such as prescribing antibiotics, planning the control of virulent diseases through vaccination and/or mass drug administration, offering genetic advice to family planning, and treating chronic conditions such as diabetes, high blood pressure, cancer, asthma, allergic reactions, and obesity. Two new disciplines have emerged from the research activities in this area: Darwinian medicine⁴ and evolutionary epidemiology^{5,6}.

The most obvious examples of evolution in action in a medical setting are perhaps the development of resistance following the use of drugs against pathogens (such as antibiotics, antiretrovirals, or antimalarials) or resistance following the use of insecticides against disease vectors⁷. Well documented additional examples are the co-evolution⁸ of the human host resistance to malaria driven by genetic selection of the sickle cell trait^{9,10} and the CCR5-Delta32 deletion allele implicated in the mechanisms of resistance against HIV-1, bubonic plague and smallpox^{11,12}. Also, the debate about the obligate evolution of the host-pathogen interaction towards commensalism represents a classic contribution to the foundations of this new discipline⁵.

Examples that compose the current research agenda of investigators in the field of evolutionary epidemiology include the impact of vaccination on the evolution of parasite virulence¹³, the emergence of new infectious diseases¹⁴ and whole-genome analysis¹⁵ of pathogen¹⁶ and vector^{17,18} evolution. This research agenda is influenced by recent advances in molecular biology and genetics that provide new tools to monitor the diversity among pathogens, vertebrate and invertebrate hosts¹⁹⁻²¹.

Public health measures to control infectious diseases have relied on the use of vaccines, insecticides and the various types of drugs and, understandingly, most of the literature cited above addresses the evolutionary implications of such control measures. New control strategies based on the genetic manipulation of pathogens and vectors, made possible by current advances in molecular genetics and mosquito ecology²², also holds a promising future and could act synergistically to current control strategies. Genetic methods for controlling vector transmission are de-

signed to reduce or eliminate vector populations, to selectively kill only those vectors infected by the pathogen, or to modify (replace) natural vector populations by introgressing genes that eliminate vector competence. Mechanisms of action of transgenes display route of entrance and tissue specificities in vectors and can, therefore, lead to distinct qualitative dynamics. These methods are at various stages of development and readiness for field-testing. The evolutionary impact of different transgenic mosquito strategies is an important component in the discussion of benefits, risks, and research priorities associated with using genetically manipulated insects in the control of vector-borne diseases^{23,24}.

In this review, we intend to introduce the basic principles of evolutionary epidemiology. We use examples of significant evolutionary insights drawn from the emerging activities on the new strategies to control vectors of parasitic and viral diseases of importance for public health. Although we are dealing with a broad subject our approach serves two main objectives. First we hope to introduce social scientists to the main questions at the interface between evolution and epidemiology. Second we intend to complement the vast literature on the subject by reviewing the main contributions to the field of vector-borne diseases having in mind the new disease control actions being proposed.

Basic principles

This review is written under the assumption that evolutionary thinking may contribute towards the alleviation of human health problems. For example, understanding the evolution of parasite virulence and drug resistance it might be possible to design better vaccines, prevent the emergence of highly virulent strains possibly induced by current public health practices, diminish the virulence of present pathogens, and improve the use of antimicrobials. The proposed framework to explain how natural selection has shaped humans and shaped the parasitic organisms with which humans interact is based on a trade-off model between transmission and virulence in which the following premises hold: parasite-caused host mortality is costly for the parasite and transmission and virulence are correlated. We visit next the main elements that shape the definition of reproductive success and virulence, two basic concepts that are central to this view.

Reproductive success

The most basic evolutionary process we are dealing with is natural selection. It is important to stress the level at which this process acts. Natural selection favors individual traits that increase the passing on of the genes that code for the traits. Therefore, there is no reason to presume that natural selection favors what is best for the species or for the greatest number of individuals over the greatest amount of time. This latter concept (that selection could act at the species level) led to a widespread misunderstanding in the past, i.e., the idea that parasites should evolve toward benign coexistence with their hosts. Parasitic strategies that lead to higher replication rates and, in this way, to a greater presence of the genes coding for this strategy in the next generation of individuals, will prevail over competing strategies, no matter their impact on the parasite host. The relevant concept here is the number of genes passed onto the succeeding generation³.

Reproductive success can be measured by the basic reproductive number (R_0). It is a measure that summarizes the tradeoffs involving all dimensions that contribute to the dynamics of a particular population of interest. It describes the number of individuals in a subsequent generation that originates from one particular individual. In the context of infectious diseases, describes the number of secondary cases generated by the index case in an otherwise uninfected host population. In general, eradication of an infectious disease becomes possible when the control measures available are successful in reducing R_0 to $R_0 < 1$.

Probably, malaria offers one of the most important examples of the application of the concept of which, in this case, is given by the expression $R_0 = \frac{ma^2bp^n}{-\ln(p)r} \times \frac{c}{r}$.

The following notation applies: m mosquito density, a biting rate and ma number of mosquitoes that bite an infective human host per day, b proportion of daily mosquito bites that are infective to mosquitoes, p^n mosquito survival probability beyond day n (extrinsic incubation cycle) after infection, c proportion of daily mosquito bites that are infective to humans, r human recovery rate, $-\ln(p)$ expected survival time once the extrinsic incubation cycle has been completed. This expression reflects the contribution of the entomological and human component to disease transmission and explains the rationale for various control measures still in place nowadays. For example, the use of larvicides or elimination of

breeding sites of the vector affect R_0 linearly through the parameter m . On the other hand, the use of insecticides that kill adult mosquitoes affect R_0 exponentially through the parameter p , the probability of daily survival. For an heuristic introduction to this expression see Ribeiro & Struchiner²⁵ and for a formal definition of R_0 see Diekmann & Heesterbeek²⁶. A historical account of the evolution of the concept of R_0 in the various disciplines and the description of a procedure for its calculation can be found in Heesterbeek²⁷.

Given the virtues of R_0 as a measure of effective transmissibility, the traditional approach of predicting evolutionary outcomes has relied on the maximization of this quantity. The criterion for the success or failure of a pathogen strain that differs in aggressiveness from a resident strain is its rate of spread through a given host population. Under the approach of maximizing a pathogen's transmissibility, if the new pathogen spreads faster than its predecessor does, i.e., if its R_0 is higher than its predecessor's, then it may invade and replace that predecessor resident pathogen. It is easily shown that the transmissibility of a pathogen can be the highest at intermediate levels of virulence and, therefore, a more aggressive pathogen strain might invade and displace a more benign resident strain. This behavior explains why pathogens and their hosts do not always evolve in ways that would render benign the consequences of infection and explains stable patterns of intermediate levels of virulence mediating the pathogen-host interaction. However, R_0 does not tell us the full story as we shall see next Dieckmann²⁸.

The limitations of the approach based on R_0 maximization are due to: (i) R_0 describes the performance of a strain in an environment of uninfected hosts only and does not describe the capacity of that strain to displace a different strain already infecting the host population; (ii) an optimization principle describing the quantity to be maximized by evolution might not exist as is the case of the of frequency-dependent selection, when selective pressures and the resultant invasion success depend on the composition of the resident pathogen population; (iii) a static optimization principle based on R_0 assumes that pathogens often have much shorter generation times than their hosts and may be expected to experience essentially a non-evolving host population in the course of their adaptation and, therefore, cannot account for the adaptation of pathogen-host interactions in terms of complex co-evolutionary dynamics; (iv) usually adaptation can only explore the small range of variation that is acces-

sible by gradual change and some evolutionary outcomes predicted by the analysis of evolutionary stability alone, such as the optimization principle based on the maximization of R_0 , actually cannot be reached by a sequence of small adaptive steps.

Given the limitations of the conventional approach to maximize R_0 , an extended framework to describe the complex processes that arise from the evolution of general pathogen–host interactions becomes necessary. The alternative approach of *adaptive dynamics*²⁸ addresses the challenges listed in the previous paragraph. This approach takes into account that the fitness of a phenotype can only be evaluated relative to the environment that this phenotype experiences, and identifies four invasibility patterns. Under *evolutionary stability* a singular phenotype is immune to invasions by neighboring phenotypes. This concept is a local version of the classic evolutionarily stable strategy (ESS) condition that arises in evolutionary game theory. ESS is an equilibrium condition which is “evolutionarily” stable, meaning that once it is fixed in a population natural selection alone is sufficient to prevent alternative (mutant) strategies from successfully invading.

Theory indicates that, contrary to what might be expected, a population does not always evolve toward an evolutionarily stable state. Evolution can lead away from an ESS and, in this case, we say that the ESS is evolutionarily unattainable or convergence unstable. *Convergence stability* is attained when invaders starting from neighboring phenotypes in relation to the resident strain do succeed and lie closer to the singular phenotype²⁹. Under this condition, any mutant allele of small effect can invade the resident population, but the mutant allele cannot entirely displace the resident allele and some form of evolutionary diversification occurs.

Mutual invasibility implies that if a pair of neighboring phenotypes lies either side of a singular phenotype they can invade each other. Coexistence of phenotypes and the emergence of polymorphisms arise as a possibility when this condition is met. The characterization of an evolutionary endpoint also benefits from a discussion of the *invasion potential* of a singular phenotype towards its neighboring types.

Virulence

It is not easy to define virulence both at a molecular level and at the level of the population. For population biologists, virulence is the in-

creased host mortality resulting from parasite infection³⁰. This definition makes clear that virulence has an unambiguous negative impact on the fitness of most parasites since, for many life cycle patterns, death of the host halts further transmission of the parasite. As expected, this definition does not please everyone since it ignores many dimensions present in parasite–host relations that harm the host and may rightly be considered virulence³⁰.

Virulence varies between strains of most pathogenic parasites suggesting the plausibility of being the outcome of evolutionary processes. However, high virulence can exist without any apparent evolutionary history in a host, as is the case of zoonotic diseases in which humans are not the natural host, e.g., yellow fever and other hemorrhagic fevers, hantavirus and arenavirus infections, viral encephalitis, and tick fevers. The trade-off model, in particular, requires a definition of virulence based on traits contributing to parasite fitness and thus being under the action of evolution. Although it is possible to trace an evolutionary component leading to virulence diversity, there are examples in which virulence has little to do with transmission and where intervention resulted in a change of virulence that was inconsistent with the classic trade-off model³¹.

The diversity of multistage life cycles of various tropical parasites points to additional aspects of the host–parasite interaction which might also mediate parasite fitness, such as the host immune response, and the time course and tissue tropisms of infection. Therefore, a more realistic view of trade-offs should include not only host mortality, but also factors potentially associated with parasite evolution as an outcome of within-host and between-host competition among parasite strains. Factors that have entered the theoretical studies so far suggest that the virulence optimum is sensitive to the abundance of susceptible hosts, the intrinsic host lifespan, the rate of clearance of the infection by the host’s immune system, the size of the inoculum, the route of inoculation, the frequency of naturally occurring infections, metabolic costs, modes of transmission (horizontally, vertically, vectorborne airborne- or sexually), pathogen genotype variation, antimicrobial dose dependence, host nutrition, age at first exposure, interactions with other infectious diseases or parasite species, the mode of action of the transmission control measure (infection blocking, transmission blocking, disease modifying, and anti-toxin), and specificities of pathogenesis (parasites that must kill their hosts to effect trans-

mission, parasites that castrate or sterilize their hosts, parasites that are virulent by means of toxins). The main message that emerges from this large body of empirical evidence is that one cannot validate the trade-off model in natural conditions because of the large number of unmeasured variables³¹.

The reason why parasites harm their hosts if a live and healthy host is beneficial to their transmission has been extensively debated in the literature, as we have pointed out. There are several difficulties with empirical applications of the trade-off model. Virulence management is based on the idea that changes in opportunities for parasite transmission will select for changes in virulence. If the correlation between transmission and virulence is low and the response to selection slow, management cannot be achieved in a timescale appropriate for guiding public health actions. Also, virulence is not always a simple function of parasite reproduction. For observing a virulence optimum as an outcome of a trade-off mechanism, virulence has to increase more rapidly than transmission rate. This condition for the existence of a virulence optimum has not been supported with empirical data. The literature does not support either the view that many infectious agents have evolved new levels of virulence in recent years. The use of vaccines and antimicrobials, and modern hygiene standards should have selected large changes in virulence if the theory was correct. Experiments conceived to reveal the causes of evolution of virulence and to test predictions of the trade-off model need to incorporate sufficient biology and control for long term trends (e.g. nutritional, behavioral, and environmental) that could confound the observed changes in virulence³¹.

Current scenarios

Recent advances in molecular biology and genetics provide new tools to monitor diversity among pathogens, vertebrate and invertebrate hosts. The genetic manipulation of pathogens and vectors also holds a promising future. We briefly review some of the current main research issues brought by these new technologies and exemplify the application of the evolutionary paradigm introduced above to this new scenario.

Molecular biology and epidemiology

Following the birth of molecular biology a

new paradigm was introduced in the epidemiologic literature in the early 80s. It had an impact in uncovering new sources of heterogeneities and illustrates the importance of taking into account the biological details of the interaction between the pathogen, the vector and the vertebrate hosts. Under this paradigm, the design of control strategies against endemic diseases started to explain the mechanism of action of the intervention itself. The three main mechanisms considered were infection blocking, disease (morbidity) modification, and transmission blocking. The discrimination among the three mechanisms was motivated by the early attempts to develop a malaria vaccine. In this context, the search for potential target epitopes for a vaccine was explicitly associated with different stages of the parasite (sporozoite, merozoite, and gamete), each stage corresponding to a mechanism of action³².

Mechanism-specific models have shown to be useful to uncover the complex implications of intervention programs against mosquito-borne diseases. Halloran *et al.*³³ focused on the changes in immune profile of the target population. Gandon *et al.*³⁴ relate the mechanism of action of a vaccine to the evolution of pathogen virulence. These authors show that leaky vaccines designed to reduce pathogen growth rate (disease modifying) can lead to higher levels of virulence that translates into more severe morbidity among the unvaccinated individuals. Based on these preliminary findings, it becomes necessary to further investigate the evolutionary impact implied by the use of antiretrovirals that lower virus load among HIV infected individuals or, for that matter, the development of vaccines that modify the course of infection caused by HIV. The epidemiological and biostatistical literature that followed clearly acknowledges this distinction and its implications. In particular, new definitions of vaccine efficacy have been proposed³⁵⁻³⁷.

Heterogeneity uncovered

Virulence is not a fixed property of infection but is affected by the genetic diversity of the players involved, i.e., the vertebrate and invertebrate hosts, the pathogen and the environmental conditions under which those players interact³⁸. Above, we introduced as a measure of reproductive success and offered a very simple expression that illustrates its use in malaria. The parameters that entered that expression can be broken down into a multitude of new parameters accounting for known additional details, each

assuming a stochastic distribution that better describes individual heterogeneities.

For example, the anatomical structures that serve as developmental sites for the various stages of a pathogen in a mosquito vector include the salivary glands, the midgut, the hemolymph and hemocoel, the peritrophic matrix, the distal cells of the Malpighian tubules, and the thoracic musculature. All these pathogen-tissue encounters within the mosquito vector represent new opportunities for vector immune responses to pathogen invasion. The biological mechanisms that mediate the vector susceptibility to the pathogen and interfere with the reproductive success of a parasite in the vector include melanotic encapsulation, phagocytosis and production of antibacterial compounds and immune peptides. Together, they represent candidate pathways through which virulence could be under the influence of natural selection³⁹.

Blood-feeding arthropods require the unimpeded flow of blood from host organisms. Antihemostatic factors present in the mosquito saliva allow these insects to blood feed efficiently. Improper functioning of these antihemostatic factors represents a potential barrier to pathogen development since it leads to coagulation of blood within the midgut and can inhibit ingested pathogens from migrating out of this environment as is required for further development. This potential barrier to pathogen development could become the target of potential control strategies and adds to the complex mechanisms that set the stage for strong selection pressure on pathogens. The biochemical makeup of the ingested blood containing the pathogen affects the parameters that enter the expression, such as, the mosquito biting rate, its mortality, and the developmental period of the parasite within the mosquito.

Regarding the heterogeneity of the parasite, the concept of *quasispecies* is particularly important in this context and applicable to the evolution of pathogens that generate high level of genetic variation, such as the RNA viruses. This concept describes the final distributions of clouds of genotypes subject to mutation-selection balance and that evolved to a stable state⁴⁰. The concept offers a novel evolutionary dynamics insight that explains the phase transition in the composition of genotypes in populations as modulated by changes in mutation rate. The theory of *quasispecies* motivated an important medical application, the extinction of RNA virus populations by lethal mutagenesis achieved by the use of the antiviral drug ribavirin. This drug contains base

analogs that are incorporated into the viral genome elevating mutation rates to the point that the virus population disappears.

Whole genome studies

The availability of the genome sequences of vectors, hosts and parasites has enabled genome wide comparative studies. Of particular interest are the studies comparing the insect immune repertoire, the means whereby those insects are able to kill invading pathogens. Knowledge about the genes controlling the susceptibility of the vector to the pathogen helps in understanding the mechanisms that mediate parasite development, from the time the pathogen is ingested in a blood meal until the infective stage of the pathogen is transmitted to the vertebrate host. This knowledge is crucial in developing new technologies of vector control by interfering with normal pathogen development within the vector⁴¹.

Large-scale bioinformatic methods, manual curation, and phylogenetic analyses of the available genomes turn it possible to identify immune signaling pathways and response modules in insect vectors. Nine sequenced genomes from four holometabolous insect orders, spanning 350 million years of evolution are the subject of attention and include the yellow fever and dengue vector, *Aedes aegypti*, the malaria vector *Anopheles gambiae*, and the fruit fly *Drosophila melanogaster*. This approach explores important evolutionary differences among the species. The two mosquito species diverged ~150 million years ago and the fly separated from them ~250 million years ago. Hematophagy to sustain abundant progeny production in mosquitoes provided a new point of entry for pathogens an aspect sharply contrasting with the fruit fly reproductive strategies¹⁸.

Using a comparative phylogenomic analysis of the insect immune repertoire, the gene families and functional groups implicated in classical innate immunity or defense functions, such as apoptosis and response to oxidative stress, recapitulate the evolutionary steps leading to the divergence or conservation of the gene subsets. These modes of immune evolution are linked to the modes of action of the immune mechanisms, such as recognition of parasite invasion and neutralization of the microbial source of the immune signal. Mechanisms acting directly on microbes diversify rapidly or are species-specific. These mechanisms suggest co-evolution with pathogens and can be evaded by them. On the other hand, effector enzymes that produce chemical cues to

attack invaders remain conserved probably as the outcome of strong selective pressure¹⁸.

New interventions

Several new intervention strategies against mosquito-borne diseases are being proposed. Their own novel mechanism of action explores the sensitivity of the dynamics of mosquito-borne diseases to genetic heterogeneities at the various stages of the parasite life cycle in the vector and in vertebrate hosts, as shown above. Genetic methods for controlling vector transmission are designed to reduce or eliminate vector populations, to selectively kill only those vectors infected by the pathogen, or to modify (replace) natural vector populations by introgressing genes that eliminate vector competence. Innovative control strategies based on transgenesis of mosquito vectors offers potentially smaller ecological and environmental impact than control strategies that rely heavily on mosquito control using insecticides, which are susceptible to the emergence of insecticide-resistant mosquito populations^{24, 42}.

Mechanisms of action of transgenes display route of entrance and tissue specificities in vectors and can, therefore, lead to distinct qualitative dynamics. They can be grouped as: strategies that block transmission, either from humans to mosquitoes or from mosquitoes to humans; reduce mosquito biting by interfering with the host-seeking behavior, perhaps only after the first blood meal or, only upon infection; raise mosquito background mortality through the release of engineered males homozygous for a dominant female-killing gene (elimination of female offspring); or raise mosquito infection-induced mortality, i.e., lethal genes only expressed in the presence of infection in mosquitoes.

These methods are at various stages in their development and readiness for field-testing. Despite the promise of these new control strategies, these interventions select for changes in pathogen virulence to both the human and mosquito hosts and their evolutionary impact remains to be explored. Under this framework, the trade-offs of importance should entertain the definition of virulence and fitness costs in both hosts as well as the pathogen simultaneously. It is possible to speculate that transgenic strategies based on blocking transmission or reducing mosquito biting could select for increased virulence to humans while strategies that increase mosquito background or infection-induced mortality do not select for changes in virulence to humans. However, it is also plausi-

ble to suppose that whether selection is to increase or decrease virulence is sensitive to the specific choices of trade-off between virulence and other epidemiological traits.

Study design

The formalism of epidemiologic methods, which has proved useful in identifying risk factors in chronic and infectious diseases as well, still requires further developments in order to guide the design of field studies to address key questions in evolutionary epidemiology. Of great need are epidemiologic and surveillance methods that could identify and monitor changes in pathogen virulence due to public health practices such as vaccination and large scale use of antivirals. In this context, the usual definitions of *efficacy* and *effectiveness* will need to follow a new paradigm and also address the evolutionary dimensions involved. The challenges ahead cannot be underestimated. It will be necessary to develop analytical models and sampling procedures that could accommodate the various levels of observation (from the molecular to the population levels), hierarchical correlation patterns (within and between hosts, within and between households and villages, within and between races), and conceive new measures of association motivated by analytical tools borrowed from other disciplines such as population genetics and phylogenetics³².

Many ecological and evolutionary consequences of long term disease control measures in populations will become obvious only on time scales longer than those of field trials required to license new vaccines, antimicrobials or genetically modified disease vectors. It is expected that large-scale use of any of those disease control strategies will alter the number and virulence of pathogen genotypes, either by reducing the force of infection or by directly altering the population dynamics of subsets of the circulating genotypes. Therefore, the licensing of new intervention tools as well as the surveillance of new and current strategies already in use must take into account the impact of control strategies on the evolution of virulence in pathogens. Analogously to current vaccine trials that provide the necessary empirical background to assess the efficacy of a vaccine, informed public health decisions in this area must rely on study designs, sampling mechanisms and epidemiologic parameters specifically conceived with this end in mind and yet to be developed. Of great importance are surveillance methods that could inform the decision making

ahead of time, much before the appearance of virulent strains becomes a reality.

Discussion

In this work, we briefly reviewed important issues revealed by the application of evolutionary theory to epidemiological problems. The scope of our work is restricted to infectious diseases and the evolution of virulence as a consequence of public health strategies to control transmission. We focused on the discussion about the possibility of virulence management and explored current scenarios in which recent advances in molecular biology and genetics provide new tools to monitor and change diversity among pathogens, vertebrate and invertebrate hosts. Vaccine-driven evolution of pathogen virulence, either by selectively acting against a subset of the antigenically diverse population of pathogens, or evolutionary changes on pathogen traits related to transmissibility allowing for immune evasion, is a related area that is receiving considerable attention more recently.

The need to integrate the analytical framework of epidemiology into population genetics, and evolutionary theory seems of paramount importance. This unified framework of analysis holds the promises of simultaneously addressing modern emerging theories at the interface of these disciplines. Following the epidemiological tradition, we anticipate as an outcome of this process the development of study designs and analytical tools to predict the evolutionary implications of control measures in the population

and surveillance mechanisms to continuously monitor the changes in pathogen virulence patterns. Precise definition of biological concepts, such as virulence and reproductive success, and the development of formal causal pathways are essential to keep complexity to a minimum and allow for comparability of model outcomes. Communication among modelers, epidemiologists and molecular biologists is essential in order to design model-driven field trials and to develop data-driven analytical tools leading to conclusive findings that can inform the public health oriented decision making process.

The evolutionary impact of transmission control through the genetic manipulation of vector competence will be difficult to assess given the multidimensional nature of the outcome. For example, mechanisms that render the vector resistant to the pathogen and that have as the site of action the salivary gland, not only interfere with the natural development of the pathogen, but also with the biting properties of the vector. The mechanisms of action involved can operate at different developmental stages of the pathogen and in different tissue sites within the mosquito. However, this is not the full story since transmission control strategies based on genetically modified organisms will possibly be used in conjunction with current and future methods, i.e., insecticides, biological control of mosquito larvae, antimicrobials, vaccines, genetic manipulation of the parasite, human behavior modification, etc. It is up to this new discipline to face the challenge and indicate the ways in which pathogen virulence could be managed in this context.

Collaboration

CJ Struchiner, PM Luz, CT Codeço and E Massad equally participated in every phase of this paper.

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